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IDENTIFIERS Military Curriculum Project

ABSTRACT

This publication, the last of three course materials in the medical laboratory technician field adapted from the Military Curriculum Materials for Use in Technical and Vocational Education series, was designed as a refresher course for student self-study and evaluation. It can be used by advanced students or beginning students participating in a supervised laboratory or on-the-job learning situation. The course is divided into four volumes with student workbooks, texts, and a supplemental handbook of reference materials for each volume. Volume 1 introduces the course and reviews chemistry and safety. Volume 2 discusses body metabolites, liver function tests, proteins, carbohydrates, and enzymes. Volume 3 includes retention and excretion tests; clearance and concentration-dilution tests; gastric analysis; chromatography, electrophoresis, and other tests performed by consultant centers; hormones; and toxicology. The final volume reviews proper microscope adjustment, critical illumination, specimen collection and preservation in urinalysis, renal anatomy and physiology, the physical characteristics of urine, microscopic examination, and chemical examination. Each of the volumes contains chapters with objectives, text, review exercises and answers to the exercises. A volume review exercise without answers is provided. (KC)

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MILITARY CURRICULUM MATERIALS

The military-developed curriculum materials in this course package were selected by the National Center for Research in Vocational Education Military Curriculum Project for dissemination to the six regional Curriculum Coordination Centers and other instructional materials agencies. The purpose of disseminating these courses was to make curriculum materials developed by the military more accessible to vocational educators in the civilian setting.

The course materials were acquired, evaluated by project staff and practitioners in the field, and prepared for dissemination. Materials which were specific to the military were deleted, copyrighted materials were either omitted or approval for their use was obtained. These course packages contain curriculum resource materials which can be adapted to support vocational instruction and curriculum development.

The National Center Mission Statement

The National Center for Research in Vocational Education's mission is to increase the ability of diverse agencies, institutions, and organizations to solve educational problems relating to individual career planning, preparation, and progression. The National Center fulfills its mission by:

- Generating knowledge through research
- Developing educational programs and products
- Evaluating individual program needs and outcomes
- Installing educational programs and products
- Operating information systems and services
- Conducting leadership development and training programs

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Military Curriculum Materials
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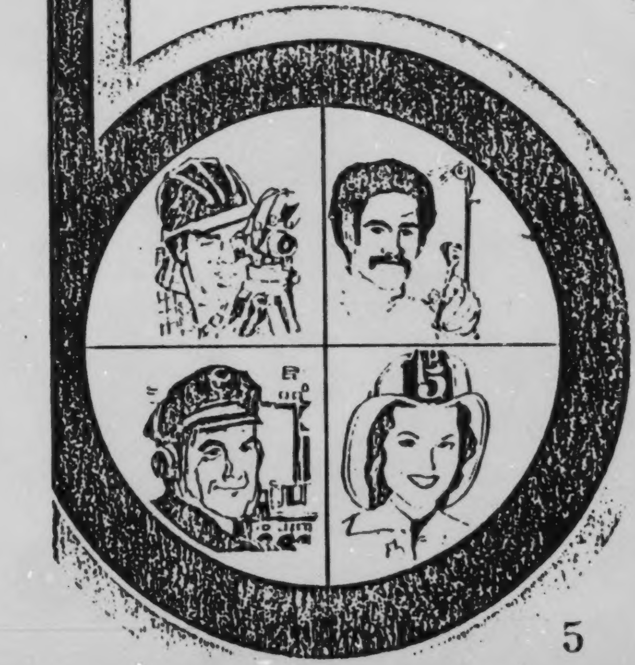
Military Curriculum Materials for Vocational and Technical Education

Information and Field Services Division

The National Center for Research in Vocational Education



Military Curriculum Materials Dissemination Is . . .

an activity to increase the accessibility of military-developed curriculum materials to vocational and technical educators.

This project, funded by the U.S. Office of Education, includes the identification and acquisition of curriculum materials in print form from the Coast Guard, Air Force, Army, Marine Corps and Navy.

Access to military curriculum materials is provided through a "Joint Memorandum of Understanding" between the U.S. Office of Education and the Department of Defense.

The acquired materials are reviewed by staff and subject matter specialists, and courses deemed applicable to vocational and technical education are selected for dissemination.

The National Center for Research in Vocational Education is the U.S. Office of Education's designated representative to acquire the materials and conduct the project activities.

Project Staff:

Wesley E. Budke, Ph.D., Director
National Center Clearinghouse

Shirley A. Chase, Ph.D.
Project Director

What Materials Are Available?

One hundred twenty courses on microfiche (thirteen in paper form) and descriptions of each have been provided to the vocational Curriculum Coordination Centers and other instructional materials agencies for dissemination.

Course materials include programmed instruction, curriculum outlines, instructor guides, student workbooks and technical manuals.

The 120 courses represent the following sixteen vocational subject areas:

- Agriculture
- Aviation
- Building & Construction Trades
- Clerical Occupations
- Communications
- Drafting
- Electronics
- Engine Mechanics
- Food Service
- Health
- Heating & Air Conditioning
- Machine Shop
- Management & Supervision
- Meteorology & Navigation
- Photography
- Public Service

The number of courses and the subject areas represented will expand as additional materials with application to vocational and technical education are identified and selected for dissemination.

How Can These Materials Be Obtained?

Contact the Curriculum Coordination Center in your region for information on obtaining materials (e.g., availability and cost). They will respond to your request directly or refer you to an instructional materials agency closer to you.

CURRICULUM COORDINATION CENTERS

EAST CENTRAL
Rebecca S. Douglass
Director
100 North First Street
Springfield, IL 62777
217/782-0759

NORTHWEST
William Daniels
Director
Building 17
Airdustrial Park
Olympia, WA 98504
206/753-0879

MIDWEST
Robert Patton
Director
1515 West Sixth Ave.
Stillwater, OK 74704
405/377-2000

SOUTHEAST
James F. Shill, Ph.D.
Director
Mississippi State University
Drawer DX
Mississippi State, MS 39762
601/325-2510

NORTHEAST
Joseph F. Kelly, Ph.D.
Director
225 West State Street
Trenton, NJ 08625
609/292-6562

WESTERN
Lawrence F. H. Zane, Ph.D.
Director
1776 University Ave.
Honolulu, HI 96822
808/948-7834

MEDICAL LABORATORY TECHNICIAN-CLINICAL CHEMISTRY AND URINALYSIS

Course Description Page 1

Volume 1

- Introduction to Your Speciality and to Clinical Chemistry - Text Material Page 3
- Workbook Page 65
- Volume Review Exercise Page 87

Volume 2

- Laboratory Procedures in Clinical Chemistry (Part I) - Text Material Page 94
- Workbook Page 146
- Volume Review Exercise Page 166

Volume 3

- Laboratory Procedures in Clinical Chemistry (Part II) - Text Material Page 173
- Workbook Page 218
- Volume Review Exercise Page 238

Volume 4

- Laboratory Procedures in Urinalysis - Text Material Page 246
- Workbook Page 299
- Volume Review Exercise Page 319

Volumes 1 through 4

- Handbook of Reference Material Page 326

Course Description:

The following course is the first of three courses in the Medical Laboratory Technician field, designed to upgrade the Specialist (skilled) worker to the Technician (advanced) level. The course contains basic information as a refresher course, but is designed to be used by advanced students or beginning students participating in a supervised laboratory or on-the-job learning situation. The remaining two courses cover Microbiology and Hematology, Serology, Blood Banking, and Immunohematology. This course lists the following duties for a Medical Laboratory Technician/Specialist:

- Performs hematological tests
- Performs urinalyses
- Performs chemical analysis
- Assists in blood bank duties
- Performs microbiological and serological tests
- Accomplishes general medical laboratory duties
- Supervises medical laboratory personnel

This course is divided into four volumes with student workbooks, texts, and a supplemental *Handbook of Reference Material* for volumes 1 through 4.

Volume 1	–	*Introduction to Your Specialty and to Clinical Chemictry* contains six chapters covering a review of basic chemistry, solutions, colorimetry and photometry, specimen collection and preparation, and quality control. Chapter 1 dealing with the introduction of the medical laboratory career field was deleted except for the section on lab safety. It covered military career organization and procedures.
Volume 2	–	*Laboratory Procedures in Clinical Chemistry* (Part I) discusses body metalbolites, liver function tests, proteins, carbohydrates, and enzymes.
Volume 3	–	*Laboratory Procedures in Clinical Chemistry* (Part II) includes retention and excretion tests; clearance and concentration-dilution tests; gastric analysis; chromatography, electrophoresis, and other tests performed by consultant centers; hormones; and toxicology.
Volume 4	–	*Laboratory Procedures in Urinalysis* covers a review of proper microscope adjustment, critical illumination, specimen collection and preservation, renal anatomy and physiology, the physical characteristics of urine, microscopic examination and chemical examination.
Supplementary Material	–	*Handbook of Reference Material* contains appendices of references on the chemical elements, clinical chemistry values, and some basic mathematics.

Each of the volumes contains chapters with objectives, text, review exercises and answers to the exercises. A volume review exercise is provided but no answers are available. This course was designed for student self-study and evaluation within the context of a laboratory or on-the-job learning situation. The material is useful for beginning students with a good science and math background or workers who wish to upgrade or refresh their skills. Much of the material is review of basic procedures with some supervisory information.

MEDICAL LABORATORY TECHNICIAN—
CLINICAL CHEMISTRY AND URINALYSIS

Correspondence Course

10-2

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Developed by:

United States Air Force

Development and Review Dates:

Unknown

Occupational Area:

Health

Cost:

Print Pages:

366

Availability:

Military Curriculum Project, The Center
for Vocational Education, 1960 Kenny
Rd., Columbus, OH 43210

Suggested Background:

Chemistry, biology, zoology, other science courses preferred

Target Audiences:

Grades 10-adult

Organization of Materials:

Student workbooks with objectives, assignments, chapter review exercises, answers, and volume review exercises; separate texts; supplemental handbook

Type of Instruction:

Individualized, self-paced

Type of Materials:			**No. of Pages**	**Average Completion Time:**
Volume 1	—	*Introduction to Your Specialty and to Clinical Chemistry*	59	Flexible
		Workbook	27	
Volume 2	—	*Laboratory Procedures in Clinical Chemistry* (Part I)	45	Flexible
		Workbook	25	
Volume 3	—	*Laboratory Procedures in Clinical Chemistry* (Part II)	41	Flexible
		Workbook	26	
Volume 4	—	*Laboratory Procedures in Urinalysis*	46	Flexible
		Workbook	25	
Handbook of Reference Material—Volumes 1 through 4			38	

Supplementary Materials Required:

None

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CDC 90411

MEDICAL LABORATORY TECHNICIAN - CLINICAL CHEMISTRY AND URINALYSIS

(AFSC 90470)

Volume 1

Introduction to Your Specialty and to Clinical Chemistry

Extension Course Institute
Air University

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Preface

THERE ARE three courses in the *Medical Laboratory Technician* specialty. The first in this course, *Clinical Chemistry and Urinalysis*. The others are 90412, *Microbiology;* and 90413, *Hematology, Serology, Blood Banking*, and *Immunohematology*. Entry prerequisites for the series are given in Chapter 1 of this volume. The prerequisite for each subsequent course (90412 and 90413) is successful completion of the previous course in numerical order as each is listed above.

The second CDC in this series, 90412, includes several unique sciences within the clinical laboratory. They are Bacteriology, Parasitology, Mycology, and Virology. CDC 90413 consideres background material in *Hematology*, the study of blood and blood-forming tissues. Another field of endeavor in this area is the study of antigen-antibody relationships, which is referred to as *Serology*. Closely allied to hematology and serology is the complex technology of *Immunohematology and Blood Banking*, a subject vital to the interests of U.S. Air Force patients.

A Handbook of Reference Material is published as a separate inclosure. Use it as the text directs throughout the course. This handbook consists of three appendixes entitled (A) The Elements, (B) Tables of Clinical Chemistry Values, and (C) Mathematics. You are not responsible for specific information in the appendixes, except when this information is referred to in the text. Appendix C is a brief summary of the basic mathematical information which you may need as a refresher for calculations in the text.

Clinical Chemistry and Urinalysis, introduces you to the USAF Medical Laboratory Service and this series of CDCs developed for your career field. Clinical chemistry is the specific chemistry of human physiology as it relates to the medical laboratory. The macroscopic, microscopic, and chemical analysis or urine is presented in the final volume of this course.

Clinical chemistry is introduced with a Review of Basic Chemistry in Chapter 2 of this volume. This is expanded in Chapter 3, Solutions, as basic chemistry principles are used to understand certain aspects of solutions used in the chemistry laboratory. The next chapter, Colorimetry and Photometry, deals with instrumentation and presents the principles and utilization of photometry in quantitative chemical analysis. Following this, the fifth chapter deals with the collection and preparation of clinical specimens. The last chapter discusses Quality Control and proposes a quality control program for the clinical chemistry laboratory.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to: Med Svc Sch (MSSTW/120), Sheppard AFB, TX 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercises, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB, Alabama 36114, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

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6 QUALITY CONTROL 49

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LIST OF CHANGES

COURSE NO.
90411

EFFECTIVE DATE OF SHIPPING LIST
23 May 75

CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.

1. CHANGES FOR THE TEXT: VOLUME 1

a. Page 7, para 2, line 17: Change this line to read "worker explain what is occurring in a particular test."

b. Page 7, col 2, line 8: Delete the comma after "oxidation."

c. Page 17, para 6-32, last equation: Add subscript "2" to "Cl" so that the equation reads "$H_2 -+ Cl_2 \longrightarrow 2HCl$."

d. Page 24, para 9-13, line 28: Change "$0.2N = C_2$" to "$0.02N = C_2$."

e. Page 31, para 11-4, line 7 from end of paragraph: Change "diulted" to "diluted."

f. Page 54, para 17-8, lines 4 and 5: Change "aliquote" to "aliquot."

2. CHANGE FOR THE TEXT: VOLUME 2

Page 7, para 1-26, line 18: Change "or" to "of."

3. CHANGES FOR THE TEXT: VOLUME 3

a. Page 22, para 5-21, line 2 from end of paragraph: Delete the square root sign.

b. Page 22, para 5-25, line 5: Change "autoanalyzer" to "Autoanalyzer®." ("Autoanalyzer" is a registered trademark.)

c. Page 25, col 1, line 19: Change "aliquote" to "aliquot."

4. CHANGES FOR THE TEXT: VOLUME 4

a. Page 17, para 7-6: Delete last two sentences containing reference to training aids package. Para 7-8, lines 1 and 2: Delete ", also included in the training aids package,".

b. Page 18, para 7-13, line 5: Change "1.002" to "0.998."

c. Page 18, para 7-15, line 10: Change "1.000" to "0.998."

d. Page 18, para 7-15, line 11: Change "1.002" to "1.000."

LIST OF CHANGES

COURSE NO. 90411

EFFECTIVE DATE OF SHIPPING LIST 23 May 75

CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.

4. CHANGES FOR THE TEXT: VOLUME 4 (Continued)

e. Page 18, col 2, para 7-15, line 15: Add as last sentence to paragraph, "The reason for adding the 0.002 to the urine reading (or water) is that the solution is less dense at 20°C than it would be at 15°C, and the calibration temperature of the urinometer used in the above situation. By correcting the 20°C reading, this is, in effect, reporting the sq gr of the urine at 15°C without lowering the temperature of the sample."

f. Page 31, para 10-9, line 2: Change "establisehd" to "established." Para 10-10, lines 11 and 12: Change "sensitiviy" to "sensitivity." Para 10-10, last line of paragraph: Change "constitutents" to "constituents."

g. Page 33, Table 3, Interpretation col, line 11: Place 2 additional asterisks (*) after "Microorganisms."

h. Page 34, para 10-16, last line: Delete "in the training aids package."

i. Page 35, para 10-21, line 8: Change "Although sulfa-para-" to "Although sulfa, para-." Para 10-22, last sentence: Change to read: "The colors are illustrated on the Labstix indicator color chart." Para 10-23, last sentence: Change to read "These directions are also on the Labstix indicator color chart."

j. Page 35, para 10-24, col 2: Change the last two sentences to read "To avoid confusion, it is suggested that the relative concentration shown on the color chart as trace, +, ++, +++ and ++++ be reported instead of reporting the specific mg % shown on the chart. This will indicate the test employed was a screening test."

k. Page 36, para 11-5, line 4: Change "if formed" to "is formed." Para 11-7, first sentence: Change to read "The directions and color range for the urine sugar test tablets are found on the package label."

l. Page 38, para 11-11, first line after the equation: Change "As you can see in" to "On." Para 11-12, lines 4 and 5: Delete the sentence beginning "The indicator is included . . ."

m. Page 40, para 12-11, lines 11 thru 13: Delete the sentence beginning "The Ictotest . . . reaction."

n. Page 41, para 12-18, last line: Change "in the Labstix indicator . . ." to "on the Labstix color chart. . ."

o. Page 42, para 12-21, line 6: Change "the HCG" to "that HCG."

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LIST OF CHANGES

COURSE NO. 90411	CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.
EFFECTIVE DATE OF SHIPPING LIST 23 May 75	

4. CHANGES FOR THE TEXT: VOLUME 4 (Continued)

p. Foldout 1, Details C and D: Reverse the captions and change "Hyatine" to "Hyaline."

5. CHANGE FOR THE VOLUME WORKBOOK: VOLUME 1

The following questions are no longer scored and need not be answered: 13 and 25.

6. CHANGE FOR THE VOLUME WORKBOOK: VOLUME 2

The following questions are no longer scored and need not be answered: 2 and 12.

7. CHANGE FOR THE VOLUME WORKBOOK: VOLUME 3

Question 28 is no longer scored and need not be answered.

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MODIFICATIONS

Pages 1-5 of this publication has (have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted material involves extensive use of military forms, procedures, systems, etc. and was not considered appropriate for use in vocational and technical education.

4. Medical Laboratory Safety Procedures

4-1. Stringent safety regulations are required in the hospital laboratory for the protection of personnel, patients, and equipment. There are many potential hazards in laboratories of which the technician must be aware. These include volatile and explosive chemicals, corrosive substances, high-voltage electrical equipment, high pressure gases (oxygen, etc.), infectious materials, chemicals which can cause systemic damage, broken glassware, and—in some cases—laboratory animals.

4-2. The laboratory supervisor is responsible for insuring that all newly assigned workers are made aware of sources of danger and means of avoiding accidents. The laboratory worker should first become familiar with AFR 92-1, *The Air Force Fire Protection Program*, and base fire regulations. A fire in a hospital can become a very tragic event. The base fire department usually conducts fire drills, and all personnel should cooperate fully in the exercises. There should be a safety shower installed so that it is readily available to everyone. The laboratory should have more than one exit. In a laboratory, safety and accuracy are inseparable.

4-3. Accuracy of results obtained in the clinical laboratory depends upon the technique of the individual performing the analysis. Good technique means more than the ability to pipette or to follow written instructions. Good techniques include proper preparation of reagents and specimens, careful treatment of glassware and other equipment, and above all, an understanding of each step in the procedures, the rationale behind each step, and the difficulties that may be encountered. Thoughtful and meticulous work is essential since the welfare of a patient may be at stake.

4-4. Your safety and that of your fellow workers in the laboratory depends upon your work habits. All spilled reagents, especially acids and bases, should be cleaned up immediately and the area should be thoroughly washed with water. If acid solutions come into contact with skin or clothing, they may be neutralized with a solution of sodium bicarbonate. Basic solutions are neutralized with a dilute solution of boric acid or acetic acid. In both cases, the area should first be thoroughly washed with water. If solutions are pipetted into the mouth, water and either dilute acetic acid or milk of magnesia solution should be used to wash the mouth thoroughly. If reagents are swallowed or splashed into the eye, or if a skin area is burned by a concentrated solution, a physician must be contacted immediately after preliminary first aid is given.

4-5. All solutions of a poisonous nature should be labeled "Poison." They should not be pipetted by mouth and should be treated with extreme care. Glassware which has contained poisonous substances should be immediately rinsed. All chemical work in which dangerous or irritating fumes are produced should be carried out under a hood equipped with an overhead exhaust fan.

4-6. Inflammable fluids must be treated with great caution. When evaporating solutions containing such liquids as alcohol or ether, use only a steam or water bath, or a closed-element hotplate. *Never* use an open-flame or open-element hotplate. Evaporation must be carried out slowly to avoid superheating which may cause an explosion. In addition to using care when handling solutions, you must also be cautious when handling glassware.

4-7. When working with glass tubing, be certain that broken edges are fire-polished. Sharp, broken edges frequently lead to accidents. If an edge cannot be fire-polished, the glassware should be discarded. When inserting tubing into a rubber stopper or cork, hold the tubing with a cloth near the point of insertion. Wetting the glass or the opening will facilitate entry. Be careful when working with hot glass.

4-8. Practice safety when handling specimens. Biological materials such as spinal fluid, blood and fecal specimens which are to be analyzed are often highly infectious. Extreme care must, therefore, be taken at all times to prevent infection. Laboratory personnel should keep their fingernails clean and short, and should wash their hands frequently with soap aand warm water.

4-9. Special precautions required in the microbiology sections and other specialized areas are covered in the respective volumes of this series which discuss these areas.

CHAPTER 2

Review of Basic Chemistry

CHEMISTRY IS THE science dealing with the composition of matter and the changes in composition which matter undergoes. Because it is such a tremendously large subject, chemistry is divided into many specialized fields—inorganic chemistry, dealing with the elements and mineral matter; organic chemistry, dealing with carbon compounds originating from living matter or from synthetic sources; physical chemistry; biochemistry; clinical chemistry; and other fields.

2. Nearly all chemistry procedures in the Air Force medical laboratory are performed in a step-by-step fashion. Actual performance of laboratory procedures is a matter of routine, with individual proficiency dependent upon a technician's ability to follow instructions and organize his work. It is possible to become quite proficient in a procedure with limited knowledge of the theory or reasoning which is involved. However, performing the procedure is only part of the responsibility of a clinical chemistry technician. In some cases, he must initiate or modify analyses. In all situations, he must be prepared to evaluate his work in terms of quality and efficiency. Sources of error are always present and must be dealt with. How can you as a laboratory worker explain what is occurring in particular test and control variables if you know only "cookbook" procedure? The answer, of course, is that you cannot effectively perform if you literally do not know what you are doing and why you are doing it. It is fundamental to understand the basic principles of chemical reactions at the atomic and molecular levels. Actual procedure should be recognized as only one essential aspect of chemistry. Mixing reagents is not an isolated skill to be developed independently of theory. A technician or chemist who has relatively little knowledge of the instructions he attempts to follow has an extremely limited potential.

3. You should have some knowledge of chemical structure and use this information to work with chemical formulas and equations. You must define the basic parts of an atom—protons, neutrons, and electrons; and you must determine the relative reactivity of an element by its configuration of electrons. You should know what determines the mass of an element and how this is related to isotopes. You should be able to describe precisely the differences in ions, molecules, compounds, and mixtures. You should be able to explain the concepts of valence, oxidation, number, chemical bonding, and polarity. With this knowledge, you can write chemical formulas and reactions.

4. In a sense, the material presented here serves as little more than an outline to encourage further study and research where needed. You will find that the material outlined in this chapter represents a deductive approach which begins with simple basic concepts, and proceeds to more complex specific procedures outlined in later chapters.

5. Atomic Theory

5-1. In the basic course, it was necessary for you to define certain essential terms in discussing atomic theory. These definitions are listed in the paragraphs below to refresh your memory for later, more advanced, discussion.

5-2. Basic units of matter are called atoms, from the Greek "atomos," which means undivided. The key to the behavior of atoms is their structure. It has long been established that matter is composed of discrete units or particles, a fact outlined with remarkable clarity in the early 1800s by John Dalton, an English teacher. Figure 2 will help you realize that the nucleus of an atom occupies a relatively small space within the whole atom. Nuclei of all atoms (except the lightest isotope of hydrogen) contain *neutrons* and *protons*. There are no neutrons in the nucleus of the lightest isotope of hydrogen. Protons are positively charged, and neutrons are neutral. *Electrons* orbit about the nucleus and are electrically negative. The negative charge of an electron is actually 1.60 times 10 to the minus 19 coulombs,[1] but it is assigned a −1 value for purposes of simplicity (1.60×10^{-19} = .00000000000000000016). A value of +1 is assigned to each proton. Electrons move about the nucleus in a path termed a ring, or orbital energy level. Further, the electrons spin in a particular way, which accounts for magnetic properties of elements. In addition to electrons, neutrons, and protons, there are other atomic particles which are not discussed here because of their peripheral relationship to clinical chemistry. Any particle found in the nucleus of an atom is referred to as a *nucleon*.

5-3. There is some difficulty in picturing an atom as having a positive nucleus and negative electrons orbiting about the nucleus. You might ask why the electrons are not attracted to the nucleus—a situation which would cause collapse of the atom. As early as 1913, scientists attempted to explain why the atom does not collapse, in terms of a quantum theory. One such theory proposed by the physicist Niels Bohr states that electrons possess a discrete quantity of energy. That is, the electrons have a specific energy level. As long as the electrons do not absorb or radiate energy, they remain at these levels. Thus, it is more correct to speak of electrons having a particular energy level than it is to speak of their occupying a particular orbit, though both terms are used. In any case, electrons do not simply collapse into the nucleus.

5-4. The maximum number of electrons which can occupy a particular energy level can be predicted by the formula, x equals $2n^2$, where x is the maximum number of electrons and n is the designated energy level counting outward from the nucleus. For example, at the first energy level or K shell, n = 1; and x, the maximum number of electrons possible, equals $2(1)^2$ or 2. The L shell, or second shell, where n = 2 and $x = 2(2)^2 = 2(4) = 8$, can have a potential population of *no more* than 8. However, as shown in figure 3, even though the 0 shell (5th energy level) theoretically could contain $x = 2(n)^2 = 2(5)^2 = 50$, it actually contains a maximum of only 32 electrons. This is because none of the known elements contains more than 32 in the 0 shell, 18 in P, and 2 electrons in the Q shell. The formula $x = 2(n)^2$ applies, then, only through the N shell. This concept is of value in the determination of valence, which we shall discuss later.

5-5. The atomic number or *Z number* of an element is the number of protons in the nucleus. There is always the same number of electrons in orbit around the nucleus as there are protons in the nucleus if the atom is electrically neutral. In neutral atoms, then, the atomic number also represents the number of orbital electrons.

5-6. The atomic mass or *A number* is equal to the sum of the protons and neutrons in the nucleus of an atom. Protons and neutrons are each assigned a mass of one atomic mass unit (amu). Electrons have approximately 1/1846 times the unit mass of a proton or neutron. This mass of the electron is considered insignificant in calculating atomic mass. One might believe that the mass of an atom could be determined exactly by counting the number of neutrons, protons, and electrons, and simply adding the masses of these basic particles. This is not the case, however, as may be illustrated with the common isotope of fluorine. It has 9 protons, 9 electrons, and 10 neutrons. For a very exacting computation of atomic mass, all of the atomic masses of the particles are added.

9 protons	(9 × 1.00759)	=	9.06831
9 electrons	(9 × 0.00055)	=	0.00495
10 neutrons	(10 × 1.00899)	=	10.08990
	Total		19.16316 amu

In applied chemistry, of course, the mass of the electron is disregarded. But even if it is not disregarded, the total mass shown above is not exactly that which is known to be correct for this isotope of fluorine. The exact mass is known to be 19.00445 amu. How do you account for the apparent discrepancy? The mass which is missing was lost when the atom was formed and is in accordance with Einstein's equation $E = mc^2$. This loss is termed the *mass defect* of the element. The Einstein equation relates mass to energy and proves that mass can be converted to energy and vice-versa. The situation is really one of conversion, not loss, and the equation or law is referred to as the *conservation of mass-energy*.

5-7. An element is the basic unit of matter that is left when matter is reduced to its simplest chemical parts. Each part, or element, cannot be subdivided or changed into any other matter by ordinary chemical means. For example, sodium chloride can be reduced to its individual elements—sodium and chlorine. It loses its identity as a salt and each element regains its original characteristics. Sodium is a highly reactive metal, and chlorine is a yellow-green highly poisonous gas. Thus, the characteristics of the individual elements differ from the characteristics of the compound they form.

5-8. Isotopes are atoms of the same element and by definition have the same atomic number, but they differ from each other in mass. Some elements have as many as 20 isotopes. In looking at the periodic chart shown in Appendix A of

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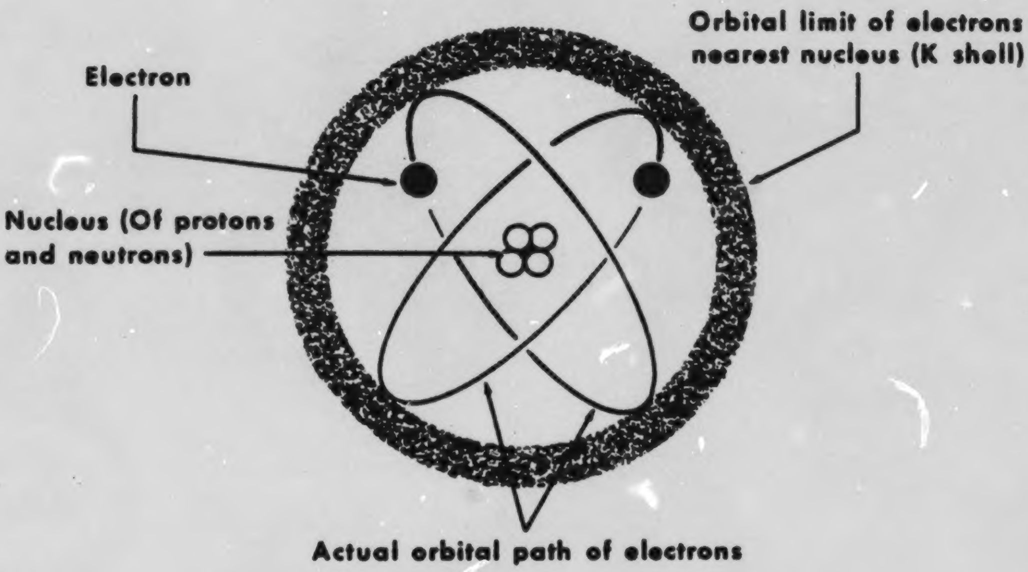
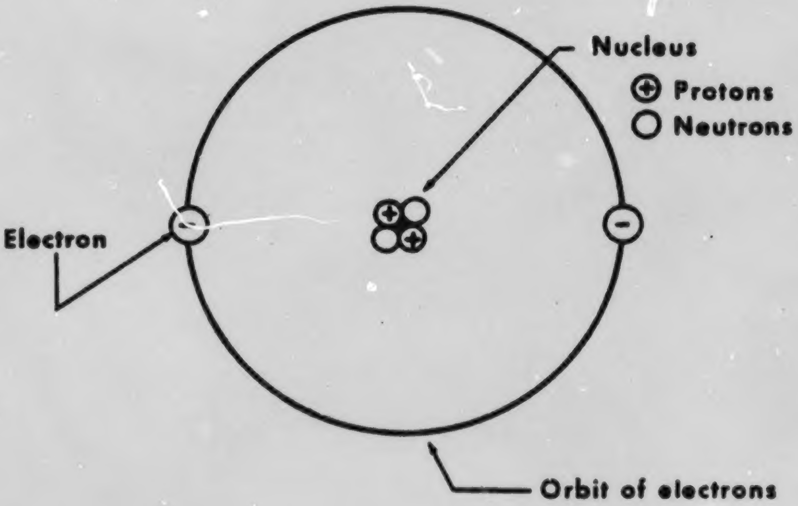
[1] The electrostatic unit of charge which is transferred by one ampere in one second is referred to as a coulomb.


Orbital limit of electrons
nearest nucleus (K shell)
Electron
Nucleus (Of protons
and neutrons)
Actual orbital path of electrons

SEMI – REALISTIC


Nucleus
⊕ Protons
○ Neutrons
Electron
Orbit of electrons

DIAGRAMMATIC

Figure 2. Makeup of the helium atom.

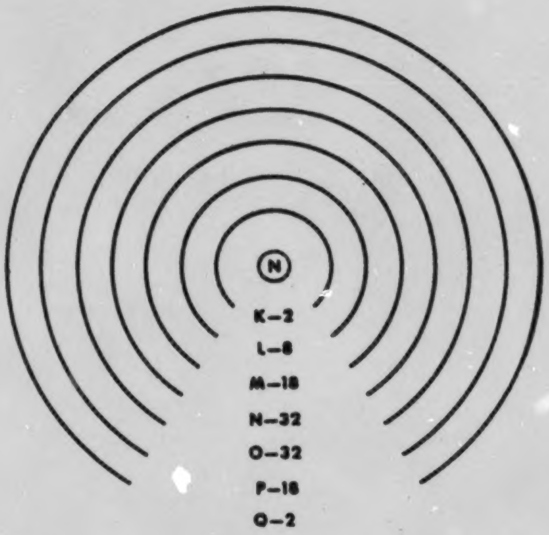


Figure 3. Energy shells by alphabetical designation.

the Handbook of Reference Material, you will notice that atomic weights are expressed in decimals. This is because many elements occur as isotopes. The weight reported on periodic charts is a mean average of the observed atomic weight, as determined by experimentation based on their occurrence in the earth's crust. Isotopes differ from each other in the number of neutrons contained in their nuclei. This accounts for the different atomic weights of atoms of the same elements. For example, there are atoms of uranium with 142 neutrons, 143 neutrons, and 146 neutrons. The result is a list of isotopes of uranium of atomic weights 234, 235, and 238. There are several more isotopes of uranium, but these are the most common. If the average of the isotope weights, based on their occurrence in nature, is taken, the atomic weight of uranium would be listed as 238.07. This is the weight given in most atomic tables.

5-9. When atoms react in chemical combination with each other, they either gain, lose, or share electrons. Any atom which carries an electric charge is termed an *ion*. When an atom loses an electron, it then has a positive charge and becomes a positive ion. An atom which gains electrons becomes a negative ion. The work required to pull an electron from a neutral atom is measured as the *ionization potential*. An atom with a high ionization potential is not easily ionized. Both ionization potential and electron affinity determine the *electronegativity* of elements, which is an expression of the ability of an atom to attract shared electrons. A scale of electronegativity values of atoms may be found in a handbook of chemistry and in most chemistry textbooks. The amount of energy released when a neutral atom gains an electron is termed the *electron affinity* of the atom. The phenomenon whereby an atom becomes an ion is termed *ionization*.

5-10. Atoms react either with their own kind or with atoms of another element. For example, one atom of oxygen can combine with another atom of oxygen to form a molecule of oxygen gas. Two atoms of hydrogen can combine with one atom of oxygen to form a molecule of water. A *molecule* is the smallest unit of a compound which retains the characteristics of that compound. When water is separated into oxygen and hydrogen, it no longer exists as water, but as the individual elements. The properties of each would be different from the properties of water. The atoms could combine with other atoms to form molecules of other compounds.

6. Combination of Elements

6-1. Most of the reagents you use in the laboratory will be made from combinations of elements called compounds. In order to use these compounds, you must know certain chemical and physical properties which govern the elements in a compound and the compound itself in its reactions. Concepts such as atomic weight, oxidation number, chemical bonding, and valence are essential for this understanding. In addition, you must be thoroughly familiar with the language of chemistry, symbols, formulas, and equations. It will be necessary for you to memorize the most common symbols and valences listed in this section. You will also require this knowledge in other chapters of this volume, so study this material so that you will retain these basic concepts and definitions.

6-2. **Compounds.** A chemical compound is a substance made up of two or more elements, chemically united in definite proportions by weight. Its properties are different from those of the elements from which the compound was formed. Table salt is a compound, made up of the elements sodium and chlorine. Water is also a compound, made up of the elements hydrogen and oxygen.

6-3. A mixture is composed of two or more elements or compounds that have been physically mixed. Unlike a compound, the typical mixture has no chemical reaction taking place between its parts. Each element or compound does not lose its original chemical properties. Generally, mixtures can be separated into individual compounds by physical means. Water added to a salt and sand mixture will dissolve the salt. By filtering the mixture we can remove the sand, and heat will evaporate the water, leaving the salt.

6-4. *Molecular composition*. Compounds have a definite molecular composition by weight. Water is an example of a compound, and it can be decomposed by electrolysis. Upon such decomposition using an electric current, it is found that 88.81 percent of the water by weight is oxygen and the remainder is hydrogen. It is a law in chemistry that the quantity of one element needed to combine with another element is a fixed ratio by weight for any given compound. This is referred to as the *law of definite proportions*. In other words, a fixed weight of one element will combine with a fixed weight of another element. Sometimes an element can combine to form different compounds in simple multiples of this fixed weight. For example, the weight of oxygen in hydrogen peroxide (H_2O_2) is twice the weight of oxygen in the compound water, but the weight of hydrogen is the same. When the law of definite proportions is expanded to explain the occurrence of elements in compounds as a simple multiple of their occurrence in other compounds, it is termed the *law of multiple proportions*.

6-5. *Atomic weight*. Expressing atomic mass in units of weight results in atomic weight. Elemental weights can be determined experimentally from the analyses of compounds. It is a simple matter to express the percent composition of a compound in terms of the percent of each element it contains. If one element, such as carbon, is given a value of weight on an arbitrary basis, it becomes easy to determine the corresponding relative weight of another element in chemical combination with carbon. This table of values has been expanded to include all of the elements, of which just over 100 are presently known. Each assigned weight is referred to as the atomic weight. For example, the compound carbon dioxide is found to be 27.27 percent carbon and 72.73 percent oxygen by weight. If the weight of carbon is 12, it would follow that in 44 g. of this compound, the weight of oxygen is proportional or present to the extent of 32 g. At this point, it is not clear if the compound contains 1 atom or 2 or more atoms of oxygen. But from the analysis of other compounds it is determined that the least weight which could combine with 12 g. of carbon is 16 g. of oxygen. Hence, the gram atomic weight of oxygen is determined to be 16 g., and the presence of oxygen in a compound to the extent of 32 g. is an example of the law of multiple proportions.

6-6. *Valence*. How elements react and the concept of compound formation is determined by *valence*. We may define valence as the number of electrons an element is able to gain, lose, or share—or simply as the combining power of an element. The valence may be either positive or negative, depending upon whether the atom is losing or gaining electrons. For example, the hydrogen atom can give up or share an electron in order to complete its valence shell. We then say that hydrogen has a valence of +1 when it gives up its 1 electron. The oxygen atom must borrow or receive 2 electrons in order to complete its valence shell. We then say that oxygen has a valence of −2. As a result, 2 atoms of hydrogen, each with a valence of +1, can combine with 1 atom of oxygen which has a valence of −2. If they combine, we will have a molecule of water.

6-7. You may not always be able to predict whether an element will gain or lose electrons, since some elements can have more than one possible valence. As a general rule, however, if an atom has 3 or less electrons in its outer (valence) ring, it will give these up, thereby doing away with this ring, making the next ring complete. (The hydrogen atom is an exception.) If there are 5 or more electrons in the valence ring, the atom receives electrons to make a total of 8 rather than giving up electrons. If an atom has 4 electrons in its valence ring, this atom has a tendency to share electrons with other atoms, rather than to gain or lose electrons. An atom with 8 electrons in its valence ring is a little more complicated. There are 6 of these elements with complete valence rings of 8 electrons. The electron distribution of the first three periods, ending with *-helium*, *-neon*, and *-krypton* is given with the periodic chart in Section IB, Periodic Table, of the Handbook of Reference Material. These are three of the 6 so-called *inert elements*, because they will not easily give up, receive, nor share electrons; consequently, they will not combine under ordinary conditions to form compounds. The valence may be determined from a knowledge of valence shell population, from knowledge of specific compounds which the elements in question form, or from a periodic chart. (See Section IB, Handbook of Reference Material.) For a list of the common elements, radicals, and their common valence states, refer to table 1. Some recent texts express valence without reference to algebraic sign.

6-8. *Oxidation number*. When valence is used with reference to reactions in which there is a loss or gain of electrons, the term "oxidation number" is more meaningful. The oxidation number has a positive or negative value, depending respectively upon whether or not electrons are lost or gained by the element in the reaction. For example, in the reaction between hydrogen and chlorine in the formation of hydrochloric acid, the oxidation number of the element hydrogen is +1 because of its loss of an electron to chlorine in the formation of HCl. The element chlorine has in this reaction acquired an oxidation number of −1. Thus, we see that the oxidation number is

TABLE 1

THE MOST COMMON VALENCES OF SOME ELEMENTS AND RADICALS

A. Positive Valences (Electron Donors)

+1	+2	+3	+4
Ammonium -- NH_4	Calcium -- Ca	Aluminum -- Al	Carbon -- C
Cuprous -- Cu	Cupric -- Cu	Bismuth -- Bi	Manganic -- Mn
Hydrogen -- H	Ferrous -- Fe	Ferric -- Fe	
Mercurous -- Hg	Lead -- Pb	Nitrogen -- N	
Potassium -- K	Magnesium -- Mg		
Silver -- Ag	Manganous -- Mn		
Sodium -- Na	Zinc -- Zn		

B. Negative Valences (Electron Acceptors)

-1	-2	-3
Acetate -- $C_2H_3O_2$	Carbonate -- CO_3	Arsenate -- AsO_4
Bicarbonate -- HCO_3	Dichromate -- Cr_2O_7	Nitrogen -- N
Halogens -- Cl, Br, I, F	Oxygen -- O	Phosphorous -- P
Hydroxide -- OH	Permanganate -- MnO_4	Phosphate -- PO_4
Nitrate -- NO_3	Sulfite -- SO_3	
	Sulfate -- SO_4	

a dynamic concept which refers to the expression of the valence potential of elements in specific compounds. For certain elements in particular compounds, the oxidation number of an element may vary. For example, iron with a *potential* valence of +3 has an oxidation number of +2 when it is in compounds such as ferrous sulfate. Since oxidation numbers depend upon how electrons are associated with the individual atoms in the various compounds, some rules may be necessary to correctly identify the oxidation number.

6-9. The rules governing oxidation number are as follows:

- The sum of the oxidation number of all atoms in a molecule is always 0.
- Oxygen usually has an oxidation number of −2 (one exception is H_2O_2, in which 7 electrons are associated with the oxygen portion of the molecule).
- In practically all compounds, metals have a positive oxidation number.
- The element hydrogen almost always has an oxidation number of +1 because it commonly forms compounds with more electronegative elements. Hydrides are an exception to this.
- The halogens usually have an oxidation number of −1 except when they are in combination with oxygen.
- Elements in the free state such as H_2, N_2, etc., always have an oxidation number of 0.

6-10. *Symbols and formulas.* The significant feature of using atomic symbols such as S for sulfur and Fe for iron is that they provide the chemist with a shorthand way to write formulas and reactions. When the chemist writes the symbol Fe, he means 1 atom of iron. The oxidation state may also be indicated by the use of + or − signs, as in using Fe^{++} to represent the ferrous ion, or +2 valence state. A subscript number after the symbol, as Fe_2, indicates the number of atoms—in this case 2 atoms. A number in front of a compound indicates the number of molecules or atoms.

6-11. It is assumed that you are familiar with the common symbols which are used to represent the elements. This information can always be obtained from a table of elements, as shown in Section IB, Handbook of Reference Material. The problem, then, becomes how to use such symbols in writing formulas. Formulas are the combinations of symbols representing compounds. Compounds are named from the formula, and the formula is derived on the basis of the valences of the 2 or more elements entering into the reaction. The valences of 2 or more elements combining chemically to form a compound determine the formula for the compound formed. The compound must be electrically neutral and, therefore, must have as many negative charges as positive. In the reaction between sodium (Na) and chlorine (Cl), the sodium ion has a valence of +1 and the chlorine ion a valence of −1. They are equal and opposite, yielding an electrically neutral compound in the ratio of 1:1. Therefore, the formula is correct as NaCl. What, then, would be the correct formula for aluminum hydroxide? Aluminum has a valence of +3 and the hydroxide radical a valence of −1. It is easy to visualize that it will take 3 hydroxides to electrically balance 1 aluminum. The formula must be $Al\,(OH)_3$. The subscript 3 following the OH in parentheses indicates that there are 3 groups. If the OH were not placed in parentheses, the 3 would appear to belong only to the H. It is very important that radicals be enclosed in parentheses when they are taken more than once. In a formula such as the one for aluminum bromide, where no radical appears, no parentheses are needed, e.g., $AlBr_3$. A convenient rule for determining what subscripts are necessary in writing formulas is the rule of crossing valences. Using zinc chloride as an example, follow the steps in the problem below.

Step 1. List the valences for the elements involved. Zinc has a valence of +2; chlorine has a valence of −1.

Step 2. Write the formula, inserting the valences.

$$Zn^{+2}Cl^{-1}$$

Step 3. Take the valence of the element at the left (Zn) and make it the subscript of the element at the right (Cl). In like manner, take the valence of the element at the right and make it the subscript of the element at the left. Thus:

$$Zn_1Cl_2$$

Step 4. Since it is understood that simply writing the elemental symbol means that 1 atom is present, the subscript 1 is omitted. Thus, the correctly written compound becomes:

$$ZnCl_2$$

This rule works for binary and tertiary compounds alike. A binary compound is formed from 2 elements; a tertiary compound is formed from 3 elements and may involve radicals, e.g., $Al(OH)_3$. If both valences are alike, as with $Ca^{++}(CO_3)^{--}$, subscript numbers are dropped and the compound is written $CaCO_3$.

6-12. It is possible to derive formulas from the gram atomic ratio of each element in the compound to the gram atomic weight of the element. For example, in 5 g. of a compound which is known to contain sodium, sulfur, and oxygen, it is found by analysis that 1.455 g. are sodium, 2.025 g. are sulfur, and 1.519 g. are oxygen. It follows that to calculate the gram atoms (i.e., the gram atomic weights) of each constituent, we divide by the respective elemental weights. The gram atoms of sodium would be 1.455 divided by 23 (gram atomic weight of sodium), or 0.063. There would also be 0.063 gram atoms of sulfur and 0.094 gram atoms of oxygen. The next step would be to find the simplest integral ratio by dividing each of these numbers by the smallest number. The result would show the following.

$$\frac{.063}{.063} = 1 \qquad \frac{.094}{.063} = 1.5$$

The simplest ratio shows 1 unit of sodium, 1 unit of sulfur, and 1.5 units of oxygen. Finally, the units must all be expressed as whole numbers. The smallest multiplier to accomplish this is 2, resulting in 2 atoms of sodium, 2 atoms of sulfur, and 3 atoms (2 × 1.5) of oxygen. The simplest formula would then be $Na_2S_2O_3$, which is sodium thiosulfate. Such a derivation is of theoretical importance in understanding how formulas can be determined from the weight of the compound and elemental composition.

6-13. *Chemical bonding.* It is acceptable to categorically describe types of bonding; however, it should be pointed out that no compound is formed exclusively by one type of bond. In *electrovalent bonding,* electrons from the valence orbit are transferred from one atom to another. A

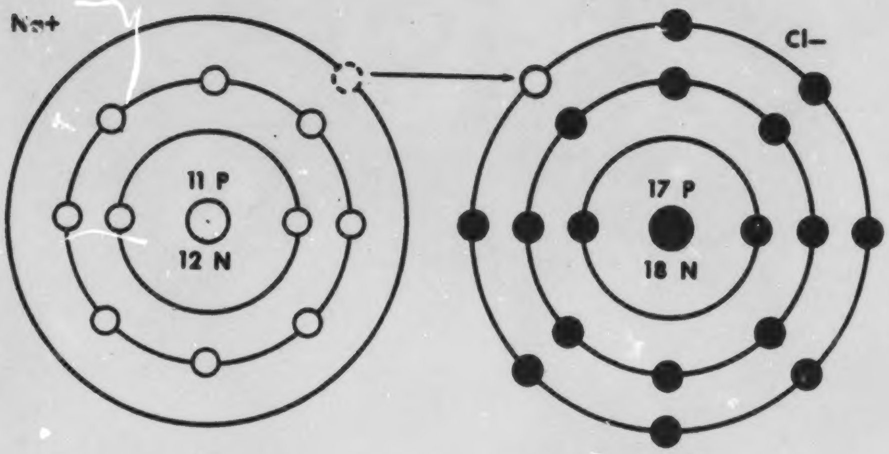


Figure 4. Electrovalent bonding in the NaCl molecule.

compound is formed from the 2 elements bound together by the electrovalent bond. This occurs in the reaction between sodium and chlorine. Sodium reacts with chlorine to yield sodium chloride (NaCl). As pointed out in figure 4, sodium has only 1 electron in its outermost orbit; chlorine has 7. In seeking stability, chlorine needs 8 electrons in the outer orbit. Note in figure 4 that the single valence electron of the sodium atom is given up and accepted into the valence shell of chlorine. The compound formed now has 8 electrons in the outer orbit and is stable. Elements which differ significantly in electronegativity values tend to form electrovalent (ionic) bonds.

6-14. In *covalent bonding* each atom donates 1 or more valence electrons to be shared equally by the two. An example of covalent bonding is the reaction between hydrogen and chlorine. Hydrogen chloride in the pure (gaseous) state is covalent, but hydrochloric acid is an electrovalent compound in water. An atom of hydrogen reacts with an atom of chlorine to yield a molecule of HCl. Figure 5 shows that the hydrogen donates 1 valence electron and the chlorine 7 to make a covalently bonded stable compound with 8 electrons in the outermost orbit.

6-15. The *coordinate covalent bond* is essentially the same as the covalent bond except that 1 atom donates all the electrons to be shared. An example of coordinate covalent bonding is represented by the compound formed from sulfur and oxygen in figure 6. In this reaction, the sulfur atom provides 2 electrons to each of 3 atoms of oxygen. But only 1 oxygen atom contributes electrons to the octet of the sulfur valence shell. A compound with 8 valence electrons in the outer rings, sulfur trioxide, is formed by coordinate covalent bonding.

6-16. The way in which charges are distributed about the center of a molecule determines polarity of the molecule. In effect, any bonding in which the sharing of electrons is unequal is a polar bond. Molecules with 2 centers of charge are called dipoles. If the 2 atoms of a diatomic molecule are alike, the molecule is nonpolar. If the 2 atoms are not alike, the molecule is polar, but if there are more than 2 atoms, it is not so simple to predict polarity. This is true because charges may cancel each other. Polarity correlates with the scale of electronegativity in that the farther apart on the scale the atoms are, the more polar the bond between them.

6-17. **Chemical Equations and Reactions.** As noted above, a chemical equation is a shorthand notation used by chemists to describe a chemical reaction. You might compare these equations with the stenographer's shorthand script since they simplify and communicate an idea. It is not necessary that an equation contain complete in-

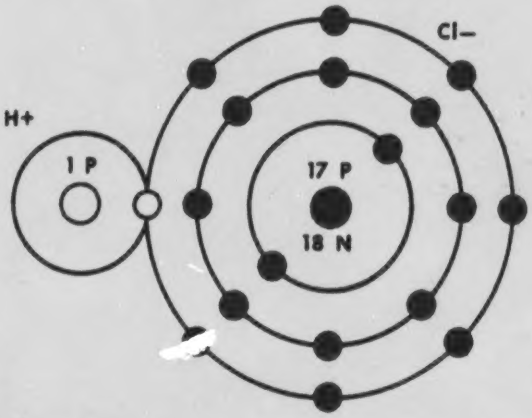


Figure 5. Covalent bonding in the HCl molecule.

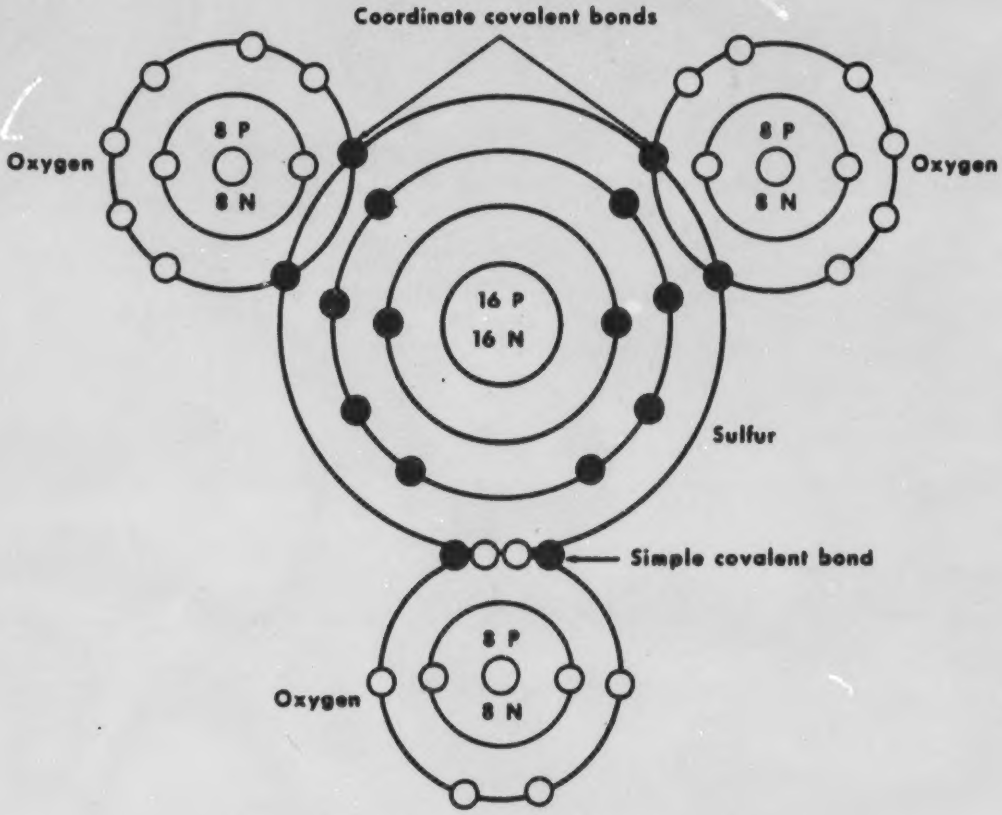


Figure 6. Coordinate covalent bonding in the SO_3 molecule.

formation about a reaction. The more common reactions may be grouped according to the products they form, and some general statements may be made concerning these types of reactions.

6-18. *Types of reactions.* The first type of reaction is the *combination reaction.* The general equation representing combination reactions is: $A + B \rightarrow AB$. A specific example of the combination reaction is the formula for a metal reacting with oxygen to yield a metallic oxide: $2Mg + O_2 \rightarrow 2MgO$.

6-19. The general equation representing *decomposition reactions* is $AB \rightarrow A + B$. The following reactions are representative examples.

$$CaCO_3 \rightarrow CaO + CO_2$$
$$Ca(OH)_2 \rightarrow CaO + H_2O$$
$$2KClO_3 \rightarrow 2KCl + 3O_2$$
$$H_2CO_3 \rightarrow H_2O + CO_2$$
$$2HgO \rightarrow 2Hg + O_2$$

The general equation for a *single displacement reaction* is $A + BC \rightarrow AC + B$. A more specific example is the reaction between zinc and copper sulfate:

$$Zn + CuSO_4 \rightarrow ZnSO_4 + Cu$$

In this type of reaction, a more active metal replaces a less active one.

6-20. The general equation for the *double displacement reaction* is $AB + CD \rightarrow AD + CB$. A more specific example is the reaction which occurs between barium chloride and copper sulfate:

$$BaCl_2 + CuSO_4 \rightarrow BaSO_4 + CuCl_2$$

This double displacement reaction is the most commonly occurring reaction. A double displacement reaction can be further subdivided into classes, of which the three most common are acid-base, precipitation, and oxidation-reduction.

6-21. An acid reacts with a base to give a salt and water. Example:

$$HCl + NaOH \rightarrow NaCl + H_2O$$

Precipitation is the throwing out of solution of a substance, usually a solid, as the result of some physical or chemical change having taken place.

6-22. In the precipitation reaction, 2 soluble substances in solution react to form 1 or more insoluble substances which precipitate from solution and settle. This is indicated in an equation by an arrow pointing downward after the formula for the precipitated material.

$$BaCl_2 + Na_2SO_4 \rightarrow BaSO_4 \downarrow + 2NaCl$$

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6-23. You have probably always thought of oxidation as the phenomenon which takes place in the rusting of iron or the burning of combustibles, when a substance chemically combines with oxygen. Actually, oxidation includes any reaction in which an atom or ion loses electrons and, therefore, gains in positive valence. Reduction is the opposite, and results in a gain in electrons and a loss of positive valence. Oxidation and reduction occur simultaneously, never separately. Let's look at some of the possible valence states of sulfur, both in its elemental state and as a compound. The valence of the sulfur is shown under the element or compound.

$$\begin{array}{cccc} \multicolumn{2}{c}{\longleftarrow \text{Oxidation}} & \multicolumn{2}{c}{\text{Reduction} \longrightarrow} \\ SO_3 & SO_2 & S & H_2S \\ +6 & +4 & 0 & -2 \end{array}$$

6-24. In the above illustration, the arrows point toward the direction of the reaction. Going from right to left (from H_2S toward SO_3), oxidation is occurring. Going from left to right (SO_3 toward H_2S), reduction is occurring. You can see that sulfur dioxide (SO_2), in going to sulfur trioxide (SO_3), presents a gain in valence or a loss of electrons, and is—according to our definition—oxidized. Going from elemental sulfur (S) to hydrogen sulfide (H_2S), there is a loss of valence or a gain in electrons, and the sulfur is reduced.

6-25. The processes of oxidation and reduction are reactions and can better be illustrated by using equations. We are considering oxidation-reduction under double displacement reactions, even though single displacement reactions are all redox reactions. (Redox is a commonly used abbreviation for reduction-oxidation.) Follow the equations below and see what happens during a single replacement reaction.

$$Zn + CuSO_4 \rightarrow ZnSO_4 + Cu$$

Elemental zinc has no charge. In the copper sulfate molecule, copper has a valence of +2 and sulfate a valence of −2, thus giving the molecule electrical neutrality. Zinc sulfate in the product combines zinc with a valence of +2 and a sulfate with a valence of −2. The elemental copper formed has a valence state of 0, since it is elemental and neutral. This can be represented in the following manner so that the whole picture is clearly visible at one time.

$$Zn^0 + Cu^{+2}SO_4^{-2} \rightarrow Zn^{+2}SO_4^{-2} + CU^0$$

Zinc has gone from a valence state of 0 to a valence state of +2.

6-26. *Writing equations.* Once the chemist knows the type of reaction that is taking place and is able to express this in symbolic terms, he is, in effect, writing an equation. Because of the basic fact that in a reaction, matter is neither created nor destroyed, equations must *balance.* That is, the total number of atoms of each element on the left must equal the total number of atoms of each element on the right. Further, the net charge on the left must equal the net charge on the right. Practice in writing and balancing equations can be achieved by referring to practically any basic chemistry textbook.

6-27. *Chemical equilibrium.* Some chemical reactions are thought to be irreversible. For example, magnesium combines with oxygen to form an oxide, but the reverse of this reaction has not been observed. Other chemical reactions are reversible, either completely or incompletely. In the case of sulfur dioxide combining with water, the formation of H_2SO_3 and the reverse reaction occur simultaneously. This is indicated by a pair of double arrows as:

$$SO_2 + H_2O \rightleftarrows H_2SO_3$$

Certain factors affect the rate of a chemical reaction and disturb the equilibrium.

6-28. LeChatelier's principle states that a system in equilibrium will react to a stress by establishing a new equilibrium. Some of the factors which may affect the equilibrium are light, pressure, temperature, and catalysts. The addition of heat will speed up a reaction only if the reaction is endothermic; i.e., absorbs heat. In other words, the equilibrium of a reaction will be shifted in the direction which absorbs heat if heat is applied. Some reactions are accelerated by the addition of light energy. It is for this reason that reagents are kept in brown bottles. The addition of pressure applies to gases, based on a corresponding volume change in the reactants or compounds formed.

6-29. The effect of catalysts is of greater interest to the clinical chemist than many other factors which affect a chemical reaction. A *catalyst* is a substance which speeds up or slows down a chemical reaction without being changed itself. Enzymes all fall into the category of biocatalysts.

6-30. In addition to the factors mentioned, the concentration of the reacting substances influences the rate of a chemical reaction. Upon reaching equilibrium, if the concentrations of the substances produced are multiplied together and divided by the product of the initial or unreacted substances, the expression is termed the "equilibrium constant." When the equilibrium constant is applied to an ionic equilibrium, it becomes the ionization constant. The value of this constant or ratio will depend upon the nature of the reacting substances, but it will be independent of the various concentrations. Besides ionization constants there may also be expressions of solubility

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product constants, hydrolysis constants, and other constants. When two reactants, A and B in a reversible reaction, form products C and D, the equilibrium constant is expressed mathematically as follows:

$$\frac{[C]\ [D]}{[A]\ [B]} = K$$

The expressions in brackets represent molar concentrations of the reactants and products of the reaction. Although values of these expressions may vary, the relationships to one another under conditions of the equilibrium remain constant. The value of using such an equilibrium constant to calculate molar concentrations is apparent.

6-31. *Acids, bases, and salts.* An acid is any substance which furnishes hydrogen ions in solution to a concentration greater than 1×10^{-7} moles per liter. A mole is defined as a gram molecular weight. (We shall discuss this area in greater detail in another chapter.) An acid is said to be strong if it is highly dissociated, and weak if it is only slightly dissociated. By dissociation we mean the phenomenon whereby atoms of a compound separate from each other as ions in solution. The Bronsted-Lowry concept emphasizes the nature of the solvent and defines an acid as a substance which can increase the hydrogen ion concentration, rather than restricting the definition to compounds which contain hydrogen ions. This broader definition includes substances which react with water to produce hydrogen. An example is carbon dioxide, as shown in the following equation:

$$CO_2 + H_2O \rightarrow H^+ + HCO_3^-$$

Another example is aluminum chloride, which furnishes hydrogen ions as follows:

$$AlCl_3 + 3H_2O \rightarrow 3H^+ + 3Cl^- + AlOH_3$$

Except in the context of the Bronsted-Lowry definition, aluminum chloride is not usually considered an acid.

6-32. Acids have the following common properties: they usually have a sour taste, affect some indicators, neutralize bases to form salts, react with some metals to form salts, react with some metallic oxides to form salts, react with carbonates to form carbon dioxide and water, and are usually (but not always) soluble in water. Generally, acids may be prepared by the following methods:

- Reacting water with a nonmetallic oxide.

$$CO_2 + H_2O \rightarrow H_2CO_3$$

- Reacting sulfuric acid with the salt of the acid.

$$H_2SO_4 + NaCl \rightarrow NaHSO_4 + HCl$$

- Direct combination.

$$H_2 + Cl^2 \rightarrow 2HCl$$

Acids may be classified in accordance with the number of hydrogen ions (protons) furnished by each molecule. Monobasic acids give 1 proton or hydrogen ion per molecule. Examples: HCl, HNO_3. Tribasic acids give 3 protons or hydrogen ions per molecule. Examples: H_3PO_4, H_3BO_4. The term "polyprotic" pertains to acids which furnish more than 1 proton per molecule.

6-33. A base is a substance containing the hydroxyl (OH) group which, when dissolved in water, forms no negative ions other than OH^- ions. Bases are proton acceptors. Sodium hydroxide ionizes in water solution to Na^+ and OH^-. The only negative ion is the hydroxyl ion. When acids and bases react in a reaction called neutralization, the proton (H^+ ion) reacts with the proton acceptor (OH^- ion) to form water. Bases have the following common properties: those that are soluble have a bitter taste, solutions feel slick and slippery like soap, and basic solutions affect some indicators, react with acids to produce salts, and react with nonmetallic oxides to form salts. Generally, bases may be prepared by the following methods:

- Some active metals react with water to form bases.

$$2Na + 2HOH \rightarrow 2NaOH + H_2\uparrow$$

- Some metallic oxides react with water to form bases.

$$CaO + HOH \rightarrow Ca(OH)_2$$

- When salts of calcium, magnesium, and iron come in contact with the soluble bases, double displacement reactions occur, forming insoluble bases which precipitate.

$$FeCl_3 + 3NaOH \rightarrow Fe\ (OH)_3 \downarrow + 3NaCl$$

- Some substances do not have an OH group but act as bases. For example, ammonia reacts with water as follows:

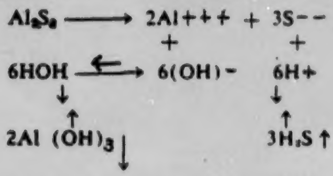
$$NH_3 + H_2O \rightarrow NH_4^+ + (OH)^-$$

Ammonia is considered a base because it furnishes hydroxyl ions. Actually, once the ammonia has reacted with water, it is no longer ammonia.

6-34. In very brief terms, a salt is formed by the combination of any negative ion except $(OH)^-$ with any positive ion except $(H)^+$. For example:

$$NaOH + HCl \rightarrow NaCl + H_2O$$

Sodium chloride is, of course, a salt. Some salts are capable of incorporating and splitting water by hydrolysis. Generally, salts formed from strong acids and strong bases do not hydrolyze, but those formed from weak acids and weak bases do hydrolyze. For example, aluminum sulfide, formed from a very weak acid and a very weak base, hydrolyzes in water solutions as follows:



$$\begin{array}{lcc} Al_2S_3 \longrightarrow & 2Al^{+++} & + \; 3S^{--} \\ & + & + \\ 6HOH \rightleftarrows & 6(OH)^{-} & 6H^{+} \\ \downarrow \uparrow & & \downarrow \uparrow \\ 2Al\,(OH)_3 \downarrow & & 3H_2S \uparrow \end{array}$$

This hydrolysis goes to completion because the hydrogen sulfide formed bubbles off and prevents the reverse reaction from occurring by removing one of the reactants. Thus, equilibrium is not established as would otherwise be expected. Composition determines whether the particular salt is acid, alkaline, or neutral. Many of the salts are white crystalline substances, but the salts of certain metals are brightly colored.

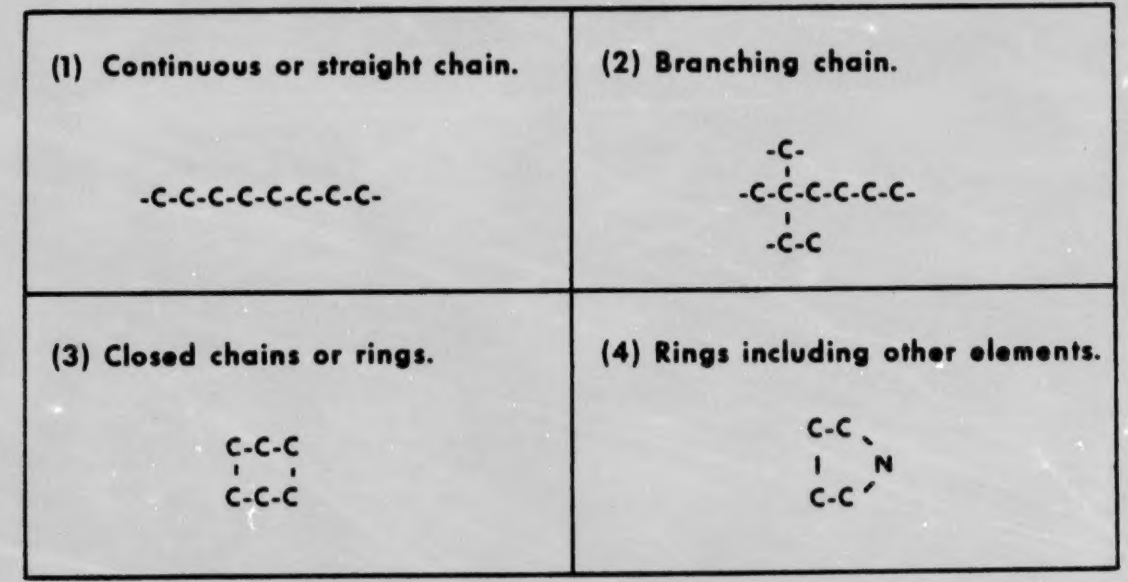
7. Organic Chemistry

7-1. Organic chemistry is the study of the compounds of carbon—compounds which are vital in the composition and function of living things. Organic substances may occur naturally, be prepared by synthesis, or may be semisynthetic—as is true of the antibiotics. Carbon possesses the unique ability to link together (bond) to form complex molecules. These molecules may form continuous chains, branching chains, closed chains, and closed chains with separating elements other than carbon. Figure 7 illustrates some of these chains. Bonds join the atoms together and determine the reactions into which the compound will be able to enter. The structure of organic compounds and the bonding they possess is best shown by graphic formulas.

7-2. Compounds composed solely of carbon and hydrogen are called *hydrocarbons*, and can be either straight-chained or cyclic. In the straight-chain group are several homologous series—that is, series of compounds differing from member to member by a common increment. By substituting an element such as chlorine for one or more hydrogen atoms in a hydrocarbon, hydrocarbon derivatives are achieved. Carbon tetrachloride, chloroform, and iodoform are important examples of derived hydrocarbons.

7-3. Aliphatic or open-chain *alcohols* consist chemically of two distinct parts: a hydrocarbon chain, often represented generally by the letter R; and one or more OH groups. Unlike the OH group inorganic compounds, the OH of alcohols is not a basic or alkaline group. Ethyl alcohol, isopropanol, glycerin, and propylene glycol are examples of alcohols. *Ethers* are dehydration products of 2 molecules of alcohols and may be either simple or mixed. Diethyl ether is a simple ether widely used as a general inhalation anesthetic, and is composed of two ethyl groups, as shown in figure 8.

7-4. *Aldehydes* are oxidation products of primary alcohols and are easily recognized by the carbonyl group and hydrogen attached to the alkyl radical. Aldehydes are usually odoriferous, ranging from very disagreeable to fragrant. Formaldehyde, paraldehyde, and chloral hydrate are examples of aldehydes. *Ketones* are oxidation products resulting from secondary alcohols and



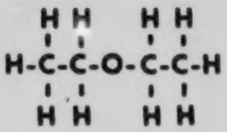
(1) Continuous or straight chain.

(2) Branching chain.

(3) Closed chains or rings.

(4) Rings including other elements.

Figure 7. Types of carbon chains.



$$\begin{array}{ccccccc} & H & H & & H & H & \\ & | & | & & | & | & \\ H- & C- & C- & O- & C- & C- & H \\ & | & | & & | & | & \\ & H & H & & H & H & \end{array}$$

Figure 8. Structure of diethyl ether.

resemble aldehydes in structure except that they have an additional alkyl radical in place of the hydrogen on the aldehyde. Acetone is a ketone; it is illustrated in figure 9.

7-5. *Esters* are products formed from the reaction between an alcohol and an acid, an acid chloride, or an acid anhydride. Cholesterol esters may be assayed in the clinical laboratory and other esters may be used as reagents. *Fats* and fixed oils are glyceryl esters of fatty acids. Waxes are closely related to fats and oils, but they have high molecular weight alcohols in place of glycerol in their structure. They are generally hard, have high melting points, and are not as greasy as fats.

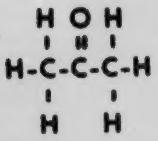
7-6. *Sterols* are high molecular weight cyclic alcohols derived from a portion of fats. Many hormones have a structure characteristic of sterols. Cyclic structures have three or more atoms joined into a closed ring. Benzene, toluene, xylene, naphthalene, and phenanthrene are basic cyclic structures. When a hydroxyl group is substituted onto an aromatic ring, the compound formed is not an alcohol, but a *phenol*. Because of the structural similarity, however, many of their properties are similar. Phenol, resorcinol, thymol, and cresol are significant phenols. Aromatic acids contain an aromatic ring and a carboxyl group. They are important mainly because of the analgesics, antispasmodics, antiseptics, and local anesthetics produced by their esterification. Aromatic halogenated compounds such as benzene hexachloride, DDT, halazone, merbromin, isodine, diiodohydroxyquin, and iodochlorhydroxyquin are generally antiseptic in nature. A few have insecticidal ability.

7-7. *Amines* and *amides*, the nitrogen-containing compounds, are very important in medicine and pharmacy because into these classes fall the alkaloids, antihistamines, sulfa drugs, barbiturates, analgesics, and local anesthetics. *Amino acids* are difunctional organic compounds containing an amino group and an acid group, either carboxyl or sulfonic. Approximately 30 amino acids are known to man. When 2 or more amino acids condense, eliminating a molecule of water, a peptide is formed.

7-8. *Proteins* are polymers of amino acids. They are an important source of food for the body and, on digestion, break down into amino acids which can be absorbed into the blood stream. *Carbohydrates* are polyhydroxy aldehydes, ketones, or organic compounds which yield these substances on hydrolysis. They are definite chemical compounds, not merely hydrated forms of carbon. Monosaccharides contain 3 or more carbons and cannot be hydrolyzed. Disaccharides are made up of 2 molecules of monosaccharides and, upon hydrolysis, separate into the 2 monosaccharides of which they are composed. Polysaccharides are more complex carbohydrates. Dextrose and fructose are monosaccharides; sucrose and lactose are disaccharides; and starch is an example of a polysaccharide. *Glycosides* are organic compounds consisting of a combination of sugars with hydroxy-containing molecules.

7-9. *Steroids* are a class of compounds found in both plants and animals. This class includes sterols and several hormones covered in Volume 3, as well as bile acids covered in Chapter 2, Volume 2.

7-10. Refer to any standard textbook of organic chemistry for a complete review of the subject and for structural formulas. We shall discuss some of the organic substances, such as carbohydrates and proteins, in some detail in Chapters 3 and 4 of Volume 2.



$$\begin{array}{ccccc} & H & O & H & \\ & | & \| & | & \\ H- & C- & C- & C- & H \\ & | & & | & \\ & H & & H & \end{array}$$

Figure 9. Structural formula for acetone.

CHAPTER 3

Solutions

YOU HAVE OBSERVED from your previous experience that solutions abound in all areas of the clinical laboratory. You should also know that while some solutions are prepared and used by any technician, certain standard solutions are carefully monitored only by supervisory personnel. Did you ever ask yourself why a particular solution was so zealously guarded?

2. Some of this mystery will be solved as you realize the nature of solutions. The importance of pure, accurate solutions cannot be overemphasized in a chemistry laboratory. In fact, the accuracy of all results obtained by a laboratory depends upon reagents which you may prepare.

3. Quality solutions are not the result of magic. They are the culmination of intelligent, exacting application of basic principles which you can learn. You must know the differences in the various percent solutions prepared in the laboratory. You must precisely define molar concentration, normality, equivalent weights, and milliequivalent weights. You should be able to explain pH and to standardize acids and bases. What is the concept of a buffer system and how does an indicator indicate? The answers to these questions are found in the basic principles of solutions set forth in this chapter.

8. Nature of Solutions

8-1. As pointed out earlier, chemical reactions involve the interaction of atoms. Any mixture in which the components are present as individual atoms or molecules is considered a solution. You can see then that as a chemistry technician you will be working with solutions. You must be thoroughly familiar with the nature of solutions and must know how to prepare the various kinds of solutions. This is a very necessary and important requirement in the chemistry laboratory.

8-2. **Terminology.** In practical terms, a *solution* is a homogeneous mixture of two or more substances. This homogeneous condition distinguishes a solution from a suspension or dispersion is which the mixture is not the same throughout. Even though some substances dissociate into their respective ions, the result is still a mixture, and solutions may be considered as such. The dissolving medium is termed the *solvent,* and the substance being dissolved is termed the *solute*. Since matter may exist as a solid, liquid, or gas, any combination of the three states of matter can be thought of as a solution if it fits the basic definition. Most of the solutions used in a clinical laboratory are solids in liquids or liquids in liquids. If a solid such as iodine diffuses into another solid, this combination may also qualify as an example of a solution. Gases can also be dissolved in a liquid to make up a solution. This particular type of solution frequently has a physiological application. It may not be readily apparent which constituent is the solute and which is the solvent—as, for example, in the case of two liquids. It has been generally held by convention that if the solution is aqueous, water is the solvent. If water is not present, the liquid present in the larger quantity is the solvent.

8-3. **Types.** There are various ways to classify solutions. One way is in terms of concentration. The amount of solute present in a given volume of solution is an expression of concentration. There are four major factors which determine how much solute will dissolve in a given volume of solution. These factors are (1) the nature of the solute, (2) the nature of the solvent, (3) the temperature, and (4) (for gases only) the pressure. In this discussion we shall not refer to pressure, since it applies only to gases. Since the nature of both solute and solvent is determined by the requirements of the problem, the common variable is that of temperature. For most purposes, we can say that at any given temperature a solution is *saturated* if it holds all of the solute it can normally contain at that temperature. The reason for inserting the word "normally" is that it is possible to use a technique which results in a supersaturated solution—that is, a solution which contains more solute than it can normally contain at that temperature. One such technique would be to heat the solution and cool it again. If the solution is undisturbed, the excess solute remaining in solution causes the solution to be supersaturated. The addition of a "seed crystal" or a scratch on the inside of the container may cause the excess solute to precipitate from solution. Heating a solution does not always increase the solubility of a solute. Sodium chloride, for example, is only slightly more soluble in hot water than it is in cold water. Finally, in describing concentration, the chemist refers to an ***unsaturated solution.*** This is obviously a solution which does not contain all of the solute it can normally contain at a particular temperature.

9. Expressions of Solution Concentration

9-1. Quantitative chemical analysis requires a variety of solutions of known concentration. How accurate these solutions must be depends upon how they are to be used. For example, a percent solution of sodium hydroxide is adequate to adjust the pH of a urine specimen, but a very precise normality of the chemical is required to standardize an acid. In this section, we shall detail the various solution concentrations used in clinical chemistry.

9-2. **Percent Solutions.** In speaking of percent solutions, you are merely expressing parts of *solute* per hundred parts of *final solution.* Obviously, the term "parts" is not specific enough. It may be expressed in weight (as grams, mg., etc.) or in volume (as liters, ml., etc.). Therefore, it is necessary to specify which of four possible conditions exist. The four possibilities are (1) weight in volume *(W/V),* (2) volume in volume *(V/V),* (3) volume in weight *(V/W),* and (4) weight in weight *(W/W).* In clinical work, weight in volume *(W/V)* and volume in volume *(V/V)* are the most common. It is generally assumed that a percent solution means a solid in a liquid if the solute occurs as a solid in its natural state. Likewise, if the solute occurs as a liquid, volume in volume is implied. This assumption, though widely applied, is something less than accurate. Acids, for example, are most often liquids, but their concentrations are seldom expressed on the basis of volume in volume. However, if percent is used in conjunction with liquid reagents, including acids, it must be considered volumetric *(V/V),* unless otherwise specified.

9-3. *Preparing V/V solutions.* By definition, concentration is expressed in terms of final volume of solution, not in terms of the amount of solvent. A 10 percent *V/V* solution is prepared by taking 10 ml. of solute and adding enough solvent to bring the final volume to 100 ml. (termed q.s. to volume). Any solution proportional to this would also be 10 percent. For example, there could be 1 ml. in a final volume of 10 ml., or 100 ml. in a total volume of 1000 ml. You need only to set up a simple ratio and proportion to prepare any other amount of a 10 percent solution. Since volume varies directly with temperature, a solution should be brought to room temperature before the final volumetric adjustment is made, provided such accuracy is required. It is interesting, though not practically significant in most situations, that miscibility or intermingling of molecules of two liquids may result in a final total volume which is less than the sum of the individual volumes. In diluting a solution to a final volume, this phenomenon is ignored. In pharmaceutical techniques it is understood that 50 percent ethanol means 50 ml. of ethanol and 50 ml. of diluent (solvent), rather than 50 ml. of ethanol q.s. to 100 ml. Such accuracy is not generally required in the medical laboratory, though it is true that the total volume in this example is a few milliliters less than 100 ml. because of molecular spacing.

9-4. *Preparing W/V solutions.* In the case of dry reagents, it is a relatively simple matter to weigh a reagent and dissolve it in a suitable solvent. When a high degree of accuracy is desired, a reagent may be desiccated and weighed with an analytical balance which can approach an accuracy of 2 mg. without technical difficulty. First, you should decide the degree of accuracy required; and second, you should prepare large enough quantities to minimize error and achieve the accuracy required. You should also be cautioned that some reagents such as picric acid cannot be dehydrated without danger of explosion. The matter of expressing units of weight per 100 parts of solution is, then, no problem. Of somewhat greater difficulty is the need to express liquid reagents in terms of weight. The most direct approach is to divide the grams needed by the weight of 1 ml. (i.e., specific gravity) to convert weight to volume. Obviously, it is easier to measure liquid by volume than to weigh it. Another aspect you must consider when determining the weight of liquid is assay value. All reagents are not 100 percent pure. To correct for this lack of purity, you will need to refer to the manufacturer's labeling and then use this assay value as a correction factor. For example, if the assay is 50 percent for a particular reagent, it will be necessary to use twice as much as you would have used if it had been 100 percent pure. In other words, it will be necessary to multiply by 100/50, or 2. In summary, a formula which can be stated as follows is used:

$$\frac{\text{Grams needed}}{\text{sp. gr.}} \times \frac{100}{(\%\,\text{purity})} = \text{ml. of liquid reagent to use}$$

Percent purity is expressed in the equation as a percent figure, not as a decimal.

Example:

How many milliliters of concentrated acetic acid (sp. gr. 1.049 and 99.5 percent purity) are needed to prepare 250 ml. of a 10 percent *(W/V)* solution?

Answer:

$$10 \times 2.5 = 25 \text{ g. needed; therefore:}$$

$$\frac{25}{1.049} \times \frac{100}{99.5} = 24 \text{ ml. of acetic acid (a liquid) are needed}$$

This type of problem is quite different from one involving simple percentage calculated on a volume basis as described in paragraph 9-3. Do you see the difference?

9-5. **Molar Solutions.** The sum of the assigned weights of each atom in a molecule represents the molecular weight, or gram molecular weight if expressed in grams. The gram molecular weight of any substance in 1 L. of solution represents a 1-molar solution. Or, we say the solution has a molarity of 1. For example, a 1-molar solution of copper sulfate ($CuSO_4$) contains 159.6 g. per liter. Occasionally, reagents contain water of crystallization, and for every molecule of the pure compound there are one or more molecules of water. To expand the example used, copper sulfate may be procured as hydrous copper sulfate ($CuSO_4 \cdot 5H_2O$). (The label on the bottle gives this information.) If you used $CuSO_4 \cdot 5H_2O$ instead of anhydrous reagent ($CuSO_4$), it would be necessary for you to weigh out one formula weight, or 249.7 g., in order to achieve a 1-molar solution. The reason for this is that 5 molecules of water for every molecule of copper sulfate contribute nothing to the number of copper ions or sulfate radicals present, but water does contribute to the weight of the reagent. Thus, we see that either 159.6 g. of $CuSO_4$ or 249.7 g. of $CuSO_4 \cdot 5H_2O$ would make a 1-molar solution of $CuSO_4$ if expressed as solute in 1 L. of final solution. The symbol for molarity is *M*. The actual number of molecules in a gram molecular weight is 6.023×10^{23}, the Avogadro number. It has been found experimentally that there are 6.023×10^{23} molecules in a gram molecule or mole, and this number has been an important constant in physical chemistry. It is known as the Avogadro constant or Avogadro number.

9-6. *Equivalent weight.* The concept of molarity is based on the weight of the molecule under consideration and says nothing of the combining capacity of the substance. A more useful concept is normality, which is based on *equivalent weight.* An equivalent weight (or gram equivalent weight, if expressed in grams) is the molecular weight divided by the total positive *or* negative valence. By the total positive valence we mean the number of replaceable hydrogen ions (or cations); by total negative valence we mean the number of replaceable hydroxyl groups (or anions). For example, a reaction involving the hydrogen ions in sulfuric acid (H_2SO_4) would require that we divide by 2, since there are 2 hydrogen ions per molecule. We may also speak of combining weight or equivalent weight of *an element,* which is the atomic weight divided by the valence. Relating this concept to the Avogadro number, the combining weight either picks up or releases 6.0235×10^{23} number of electrons. If, as an example, aluminum with a valence of +3 reacts with oxygen, each atom of aluminum will lose 3 electrons. In a 1-molar solution, 3 times the Avogadro number of electrons would be released. Thus, the weight of aluminum which would release $\frac{1}{3}$ this number of electrons is $\frac{1}{3}$ the gram atomic weight. Keep in mind that equivalent weight is always expressed with respect to one of the ions or radicals. In the case of a solute which furnishes only two kinds of ions, the number of positive ions equals the number of negative ions. If more than two kinds of ions are furnished, it is important that it be specified which ion is being referred to, based on the particular chemical reaction. For example, disodium ammonium phosphate ($Na_2NH_4PO_4$) dissociates to furnish 2 sodium ions, 1 ammonium ion, and 1 phosphate radical. Thus, the equivalent weight with respect to the sodium ion is ½ the molecular weight. In the case of the ammonium ion, the equivalent weight is the same as the molecular weight of the salt because NH_4 has a valence of +1. In spite of this obvious situation, of which double decomposition is an example, you may be inclined to believe that equivalent weight (and hence normality) has no relationship to the nature of the reaction. Just the opposite is true; equivalent weight is a concept which takes into account the combining capacity with reference to a particular ion. As a final example, we might consider the equivalent weight of ferrous sulfate ($FeSO_4$). In oxidation-reduction reactions, its equivalent weight is the same as its molecular weight. In double decomposition reactions the equivalent weight is ½ this amount. How significant is this rather confusing point to you as a medical laboratory technician? Ordinarily, you are dealing with acids and bases wherein it is obvious that only two kinds of ions are furnished. But you need to be aware of equivalent weight as an expression of combining weight for purposes of the particular reaction in which the chemical is to be employed.

9-7. *Normal solutions.* Just as 1 g. molecular weight per liter represents a 1-molar solution, so does 1 g. equivalent weight per liter represent 1-*normal* solution. Normality is expressed by a capital *N*. In common usage, *N*/2 means ½ normal, *N*/10 means $\frac{1}{10}$ normal, etc. The reason for using the concept of normality is already apparent to you. A 1-molar solution of sulfuric acid (H_2SO_4) contains 2 atoms of hydrogen per molecule, and will furnish twice as many hydrogen ions as hydrochloric acid (HCl). Hence, a 1-molar solution of sulfuric acid represents a 2-normal solution, whereas a 1-molar solution of HCl is also a 1-normal solution. Do you see why?

> *Example:*
>
> How many milliliters of phosphoric acid (H_3PO_4) are needed to prepare a 1-normal solution, using phosphoric acid reagent of specific gravity 1.8 and assay value of 80 percent?
>
> *Answer:*

$$\text{Grams needed} = \frac{\text{molecular wt.}}{\text{valence}} = \frac{98}{3} \text{ or } 32.7 \text{ g.}$$

Using the formula given in paragraph 9-4, we find $\frac{32.7}{1.8} \times \frac{100}{80}$ would give the milliliters of phosphoric acid to be diluted to a volume of 1 L. for a 1-normal solution. As previously stated, the term "valence" in the denominator is synonymous with the number of acidic or ionizable hydrogen ions per molecule.

9-8. Problem Situation No. 1: Your supervisor asks you to prepare 500 ml. of a 2*M* solution of NaOH. How many grams of 100 percent chemically pure (CP) NaOH would be necessary?

9-9. Problem Situation No. 2: Your supervisor gives you an assignment to prepare 1 L. of a 2*N* solution of H_2SO_4. You find a bottle of sulfuric acid with a specific gravity of 1.834 and 98.0 percent purity. How would you prepare this solution?

9-10. Solution to Problem Situation No. 1: First, you find the gram molecular weight of NaOH by adding the atomic weights from the periodic table for each atom in the compound.

$$\begin{array}{r} 22.997 \\ 16.000 \\ 1.008 \\ \hline 40.005 \end{array}$$

Thus, 40.005 is the gram molecular weight. To make a 2*M* solution you would dissolve twice this weight, or 80.0 g. in 1 L. of water. Since 500 cc is ½ L., you can divide the weight needed for a whole liter by 2, giving 40 g. to be dissolved in 500 cc of water.

9-11. Solution to Problem Situation No. 2: Since normal solutions are based on equivalent weights, we must find the gram equivalent weight of H_2SO_4. Again, we add the atomic weights for each atom in the compound.

$$\begin{array}{r} 1.008 \\ 1.008 \\ 32.066 \\ 16.000 \\ 16.000 \\ 16.000 \\ 16.000 \\ \hline 98.082 \end{array}$$

By the rules of significant figures, round to 98.0. Dividing by the number of hydrogen ions per molecule, we have

$$\frac{98.0}{2} = 49.0$$

Thus, 49.0 is the gram equivalent weight of the acid. To make a 2*N* solution, we can use a formula like this:

$$\frac{\text{Normality desired} \times \text{gram equivalent weight}}{\text{specific gravity}} \times \frac{100}{\text{percent purity}} = \text{milliliters for 1 L. of desired normality solution}$$

Substituting in this formula, we find:

$$\frac{2 \times 49.0}{1.83} \times \frac{100}{98.0} \text{ OR } \frac{100}{1.83} = 54.6$$

Thus, to make a 2*N* solution, you would dilute 54.6 ml. of the concentrated acid to 1 L.

9-12. **Diluting Solutions.** Whenever it is necessary to prepare a dilute solution from a more concentrated one, the following formula holds true:

$$V_1C_1 = V_2C_2$$

Where

V_1 is the volume of the solution to be diluted,
C_1 is the concentration of the solution to be diluted,
V_2 is the volume of the diluted solution, and
C_2 is the concentration of the diluted solution.

As suggested earlier in paragraphs 8-3 and 9-2, concentration times volume is a means of expressing the amount of solute present. For example, in 100 ml. of a 10 percent *W/V* solution, there are 10 g. of solute. Diluting this solution merely increases the volume and proportionally decreases the concentration. *The amount of solute does not change.* If the volume were increased to 1 L., the concentration would be decreased to 1 percent, since the amount of solute is still 10 g. It should be noted that this formula holds true, regardless of the terms used to express concentration, so long as C_1 and C_2 concentrations are expressed in the same terms (mg./ml., g./L., etc.).

9-13. The next point involves understanding how to use the *V-C* formula. Concentration can be expressed in any terms, including those of percent and normality. With this in mind, let us look at some more example problems.

Example:

How many milliliters of 100 percent ethanol (absolute alcohol) are required to prepare 800 ml. of 30 percent?

Answer:

Fill in the values as follows:

$$V_1 = ?$$
$$C_1 = 100\%$$
$$V_2 = 800 \text{ ml.}$$
$$C_2 = 30\%$$

Then:

$$V_1 = \frac{800}{100}\,(30) = 240 \text{ ml.}$$

Example:

If 200 ml. of 0.1*N* sulfuric acid are diluted to 1 L., what is the resulting normality?

Answer:

$$V_1C_1 = V_2C_2$$
$$V_1 = 200 \text{ ml.}$$
$$C_1 = 0.1N$$
$$V_2 = 1000 \text{ ml.}$$
$$C_2 = ?$$
$$(200)\,(0.1) = (1000)C_2$$
$$0.2N = C_2$$

9-14. **Milliequivalent Weight.** You know from previous sections dealing with equivalent weight and normality that equivalents are related to chemical reactivity in a definite proportion. In fact, one chemical equivalent has the same chemical reactivity as any other chemical equivalent. In physiological chemistry, this concept is useful for calculations involving electrolytes and acid-base balance. When expressing the concentration of body electrolytes and in dealing with dilute solution, the clinical worker uses milliequivalent weights (mEq wt.) rather than equivalent weights. The concentrations of electrolytes in body fluid are normally quite low so that mEq/L. is a practical unit. An mEq weight is $\frac{1}{1000}$ of the equivalent weight or is equal to the millimolecular weight divided by the valence. In the case of sodium, the molecular weight is 23; the equivalent weight is also 23 because

$$\frac{\text{Molecular weight}}{\text{valence}} = \text{equivalent weight}$$

$$\frac{23}{1} = 23$$

The mEq weight is also 23. The equivalent weight and mEq weight have the same numerical designation because equivalents relate to reactivity or combining capacity rather than to weight alone. This can be more completely understood by converting a weight term, 310 mg./100 ml. of sodium, to mEq/L. The following formula will make this conversion:

$$\frac{\text{mg. \%} \times 10}{\text{mEq wt.}} \text{ or } \frac{\text{mg./L.}}{\text{mEq wt.}} = \text{mEq/L.}$$

$$\frac{310 \times 10}{23} = \frac{3100}{23} = 134.8 \text{ mEq/L.}$$

Sodium (Na), potassium (K), and calcium (Ca) are electrolytes usually reported in mEq/L.

9-15. For purposes of consistency, carbon dioxide and chloride (as NaCl) are frequently reported in mEq/L. along with the other electrolytes. To calculate CO_2 in millimoles/liter (mM/L.) or mEq/L., we divide volumes percent (vol. %) by 2.226. You will remember that 1 mole of an ideal gas occupies 22.4 L. at standard conditions of temperature and pressure (0°C. and 760 mm. Hg). Therefore, 1 millimole (mM) of this gas will occupy 22.4 ml. at standard conditions, or 22.4 ml. of CO_2 is equivalent to 1 mM. Since the test result is in vol. % (ml./100 ml.), it is also in terms of 0.1 mM. If 1 mM occupies 22.4 ml., then 0.1 mM occupies 2.24 ml. The CO_2 extraction is from an aqueous phase which introduces some water vapor to the gas. In addition to this, there is not complete extraction of the gas and a part of it is reabsorbed into the aqueous phase. All of these factors lower the amount of gas in 0.1 mM to 2.226. This, then, is the derivation of the conversion factor for changing vol. % to mM/L. or mEq/L. We shall discuss the gas laws in more detail in Chapter 1, Volume 2. In the case of chloride, as with any other nongaseous element, multiplying the mg-% figure by 10 would result in milligrams per liter. Then, dividing by the milliequivalent weight of the element in milligrams yields the number of milliequivalents per liter. A difference noted is that chloride is always reported as milliequivalents of sodium chloride, which has an equivalent weight of 58.5, not 35.46, which is the equivalent weight of chlorine. For example, a value of 350 mg-% of chloride (as sodium chloride) is equal to $\frac{350 \times 10}{58.5}$ or 59 mEq/L. The same reasoning may be applied to other inorganic elements if desired, keeping in mind that respective equivalent weights differ. Actually, inorganic phosphorus presents a problem in this regard. It exists in the serum as HPO_4^{--} and $H_2PO_4^{-}$, with about 20 percent of the latter. For this reason, the atomic weight is usually divided by 1.8, not by 2 to obtain the equivalent weight of phosphorus. This factor of 1.8 corrects for the fact that phosphorus exists in the buffer forms mentioned, while at the same time it converts atomic weight to equivalent weight. The fact that phosphorus exists in more than one valence state need not enter into the calculation if the result is reported in mg-% or mM. In order to report the result in millimoles per liter, the mg-% value is multiplied by 10, and then divided by 31, which is the atomic weight of phosphorus.

10. Hydrogen and Hydroxyl Ion Concentration

10-1. You have had some experience with the hydrogen ion concentration and buffer systems. The routine urinalysis always includes a pH determination. As you know, standard acids and bases are indispensible reagents in any chemistry laboratory. Also, because of the essential relationship of pH and buffer systems to the study of enzymes, you must understand these factors to appreciate an enzyme system. Urinalysis, standard acids, and buffers are not unfamiliar terms to you. It remains, then, for you to become more familiar with some of the implications of these terms. This section will define pH, standard acids and bases, and buffer systems and discuss some of the relative considerations.

10-2. **pH.** The term "pH" is taken from an expression which literally means presence of hydrogen. This, in effect, is a definition of pH, for it is an indication of the hydrogen ion concentration of a solution. As a means of relating changes in hydrogen concentration in small meaningful numbers, the Danish scientist, Sorensen, devised the pH scale in use today. This scale runs from 0 to 14, a pH of 14 being one in which the hydrogen ion concentration is 1×10^{-14} moles per liter. A pH of 7 is considered neutral, because the concentration of hydrogen ions which pure water furnishes upon dissociation is 1×10^{-7} moles per liter.[3] Table 2 shows the pH scale in relation to the moles per liter ion concentration. In mathematics, an exponent to the base 10 is a logarithm. It is, therefore, also possible to write pH as a minus log of the hydrogen ion concentration, or in symbols $pH = -\log [H^+]$. Remember that concentrations inclosed in brackets symbolize moles per liter. A knowledge of logarithms makes it possible to convert pH to moles per liter, or moles per liter to pH.

TABLE 2

pH SCALE RELATED TO ION CONCENTRATION

pH	H^+ Ion Concentration	OH^- Ion Concentration
0	1×10^{-0}	1×10^{-14}
1	1×10^{-1}	1×10^{-13}
2	1×10^{-2}	1×10^{-12}
3	1×10^{-3}	1×10^{-11}
4	1×10^{-4}	1×10^{-10}
5	1×10^{-5}	1×10^{-9}
6	1×10^{-6}	1×10^{-8}
7	1×10^{-7}	1×10^{-7}
8	1×10^{-8}	1×10^{-6}
9	1×10^{-9}	1×10^{-5}
10	1×10^{-10}	1×10^{-4}
11	1×10^{-11}	1×10^{-3}
12	1×10^{-12}	1×10^{-2}
13	1×10^{-13}	1×10^{-1}
14	1×10^{-14}	1×10^{-0}

10-3. *Indicators.* Chemicals which vary in color, depending upon the hydrogen ion concentration, are known as indicators. Note in table 3 that each of the various indicators has a pH range over which it has a characteristic color. If several such indicators are incorporated in a paper strip, the result is pH paper commonly used in chemistry laboratories. Individually, indicators are useful in that if the pH at which a color change occurs is known, an indicator can be selected to signal that point. For example, diethylaminoazobenzene (Topfer's reagent) is red below a pH of 2.8 and salmon pink at a pH of 2.8 to 3.0. Above this it becomes yellow. Hence, Topfer's reagent is useful in gastric analysis, discussed in a later chapter. The application of electrometric measurement is discussed in another chapter.

10-4. *Buffers.* A buffer is a solution which does not change pH upon the addition of significant quantities of hydrogen or hydroxyl ions. We frequently say buffers resist a change in pH. But how, exactly, is a buffer able to do this? The answer depends upon the very nature of a buffer. It is composed of a weak acid or a weak base and the salt of that acid or base. An example of a buffer is acetic acid and sodium acetate.

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[3] 1×10^{-7} is also the concentration of hydroxyl ions as a dissociation product of pure water. Since the hydrogen and hydroxyl ion concentrations are equal, the pH is neutral.

TABLE 3

DATA ON INDICATOR SOLUTIONS

Indicator	pH Range	Acid to Base Color Change	How to Prepare
Bromcresol green	3.8 - 5.4	Yellow to green	0.1 Gm., 2.9 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Bromcresol purple	5.2 - 6.8	Yellow to purple	0.1 Gm., 3.7 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Bromphenol blue	3.0 - 4.6	Yellow to blue	0.1 Gm., 3 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Bromthymol blue	6.0 - 7.6	Yellow to blue	0.04 Gm. in 100 ml. of absolute alcohol
Congo red	3.4 - 4.5	Blue to red	0.5 Gm., 90 ml. water, 10 ml. 95% alcohol
Chlorophenol red	5.0 - 6.6	Yellow to red	0.1 Gm., 4.8 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Cresol red	7.2 - 8.8	Yellow to red	0.1 Gm., 5.3 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Litmus	4.5 - 8.3	Red to blue	
Methyl orange	3.0 - 4.4	Red to yellow	0.1 Gm. in 100 ml. water
Methyl red	4.4 - 6.2	Red to yellow	0.05 Gm. in 100 ml. 50% alcohol
Neutral red	6.8 - 8.0	Red to yellow	0.5 Gm. in 300 ml. 95% alcohol
Phenol red	6.8 - 8.4	Yellow to red	0.1 Gm., 5.7 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Phenolphthalein	8.3 - 10.0	Colorless to red	0.1 Gm. in 100 ml. 50% alcohol
Thymol blue	1.2 - 2.8	Red to yellow	0.1 Gm., 4.3 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Thymol blue	8.0 - 9.6	Yellow to blue	0.1 Gm. 4.3 ml. N/20 NaOH, q.s. to 250 ml. with dist. H_2O
Topfer's reagent	2.9 - 4.2	Red to yellow	0.5 Gm. in 100 ml. 95% alcohol
Thymolphthalein	9.3 - 10.5	Colorless to blue	0.1 Gm. in 100 ml. absolute alcohol

If a strong acid is added to the mixture, some of the acetate ions will combine with the hydrogen ions to form acetic acid, a weaker acid. If a base is added to this buffer, some of the acetic acid will shift to furnish acetate ions and water. Graphically, the following represents a buffer system.

$$Na^+ + Ac^- + HAc \longleftrightarrow Na^+ + H^+ + 2Ac^-$$

The addition of hydroxyl ions or hydrogen ions results in equations (a) and (b) respectively.

(a) $(OH)^- + HAc \longrightarrow Ac^- + H_2O$
(b) $H^+ + Ac^- \longrightarrow HAc$

10-5. A buffer is most efficient when the concentration of the acid is equal to the concentration of the salt. This point is referred to as the pKa value of a particular buffer and is a characteristic of the buffer. In other words, at this point the addition of hydrogen or hydroxyl ions would produce the smallest change in pH, as shown in figure 10. As you can see in this figure, the midpoint of equal molar concentrations of a weak acid and salt is indicated as 1.0. It is impossible to overemphasize the significance of buffers. At least a basic knowledge of what buffers are and how they function is essential both in the laboratory and in the study of physiology. Many, if not all, biological reactions will not occur if the pH is uncontrolled by a buffer system. Buffers also maintain the stability of reagents on the laboratory shelf. Some indicators may affect the pH of an unbuffered dilute solution. In such cases, electrometric methods are preferred over indicators. If a pH meter is unavailable and the solution is not buffered, it is desirable to adjust the pH of the indicator to the approximate pH of the solution being measured to minimize the effect of the indicator on the unbuffered solution.

10-6. **Standardizing Acids and Bases.** Obviously, you need to have a known standard from which to calculate unknowns. Assuming that you are careful and accurate in your work, only one standard (called a primary reference standard), either acid or base, is necessary to determine the exact normality of all other acids and bases. A sufficient quantity of 1.0*N* acid or base can be accurately diluted to 0.1*N* and 0.01*N* to satisfy most requirements in the clinical laboratory.

10-7. *Preparation.* Standard acid or base may be secured from a reliable commercial source, though some laboratories prefer to prepare their own. One solution commonly prepared as a standard is 1*N* oxalic acid. Oxalic acid is available as a pure crystalline substance which can be conveniently weighed and dissolved in pure distilled water. It is advisable to use triple distilled deionized water, which is the kind used for injection purposes, if there is any doubt about the neutrality or purity of the distilled water available in the hospital laboratory. Compounds often used as acid primary standards may be seen in table 4. All chemical compounds cannot be assumed to be pure enough to use in preparing a standard. Sodium hydroxide, for example, contains significant amounts of impurities, including sodium carbonate. The exact normality of sodium hydroxide is determined by titration rather than by calculation of weight. For information regarding approximate normality of acid and base reagents, see table 5. In many procedures, particularly bicarbonate titration, the normality must be exact. It is well for a technician to be able to distinguish situations in which the normality of a reagent is critical from a situation such as the BSP determination, where the acid or base used need not be exact. In this way, valuable time will not be wasted attempting to achieve unnecessary accuracy. All standardized acids and bases should be labeled with the date prepared, and should be

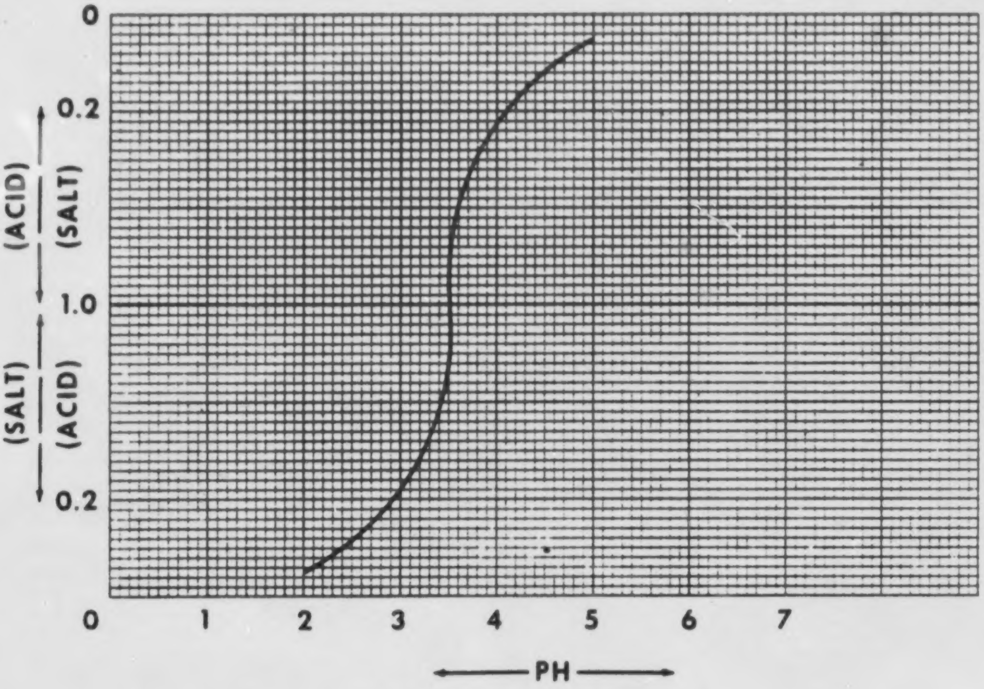

Figure 10. Approximate efficiency curve for a buffer of pKa = 3.5 pH units.

TABLE 4

SOME OF THE ACIDS USED AS PRIMARY STANDARDS

Primary Standard for Bases	Molecular Weight*	Gram-Equivalent Weight
Potassium acid phthalate $C_6H_4(COOH)COOK$	204.22	204.22
Oxalic acid $(COOH)_2 \cdot 2H_2O$	126.07	63.035
Potassium bitartrate (COOH· CHOH· CHOH· COOK)	188.176	188.176

*Always refer to label on reagent bottle.

checked as often as necessary. Whenever a sediment appears in the bottle, or if contamination is suspected, the reagent must be evaluated, and it is quite likely that it must be replaced. A common source of contamination results from the use of a contaminated pipette. Other common sources of contamination are created when stoppers are switched and when stoppers are placed on dirty surfaces. The philosophy that reagents are acceptable as long as controls are within range is not sound. We shall explain this more fully in Chapter 6 dealing with quality control.

TABLE 5

APPROXIMATE NORMALITY OF CONCENTRATED ACIDS AND BASES

Concentrated Acid or Base	Approx. Normality	Ml. Required to Prepare 1 L. of a 1 N. Solution
Acetic acid	17.4	58
Ammonium hydroxide	14.8	68
Hydrochloric acid	12.1	83
Lactic acid	11.4	88
Nitric acid	15.7	64
Perchloric acid	11.6	87
Phosphoric acid	44.0	23
Potassium hydroxide (saturated at 25°C.)	13.5	75
Sodium hydroxide (saturated at 25°C.)	19.1	53
Sulfuric acid	36.0	28

10-8. *Titrations*. The process of neutralizing an acid with a base, or a base with an acid, for the purpose of determining the concentration of one of them, based on a known value for the other, is an example of a titration. This is not necessarily a complete definition, because some titrations do not involve acids and bases. For example, in the chloride procedure of Schales, sodium chloride is titrated with mercuric nitrate. However, the principles involved are essentially the same for all titrations. An indicator is used to signal the point at which neutralization has taken place. This is termed the endpoint. It is reasonable that if it requires 5 ml. of an acid of known concentration in terms of equivalent weight to neutralize 5 ml. of an unknown base, the concentrations are equal. This is true because of the *law of combining weights*, which states that one equivalent weight combines with one equivalent weight. The formula applied is as follows:

Normality of acid × volume of acid = normality of base × volume of base at the endpoint

Example:
10 ml. of an acid will neutralize 20 ml. of an 0.1N base. What is the normality of the acid?
Answer:

$$10x = (20)\ (0.1)$$
$$x = 0.2 \text{ normal acid}$$

10-9. Phenolphthalein is commonly used as the indicator. The base is most often titrated into the acid with a burette or pipette rather than the acid into the base. This is done because phenolphthalein is colorless in acid solution, and it is considered easier to titrate to a color than to the disappearance of a color. Actually, the titration can be performed either way. Keep in mind that concentration must be expressed in terms of normality in the titration formula rather than in molarity. Do not confuse this formula with the *V-C* formula used in preparing solutions of lesser concentration from solutions of greater concentration.

CHAPTER 4

Colorimetry and Photometry

YOU HAVE REVIEWED basic chemical and physical principles in the first three chapters of this volume. Before continuing to more advanced concepts of physiological chemistry, you should have a basic understanding of the primary instrument used in quantitative chemical analysis. This instrument is, of course, the spectrophotometer. This chapter begins with a discussion of the concept of colorimetry or, literally, the measurement of color. In the not too distant past, color was measured or, more properly, estimated, with the rather jaundiced eye of the beholder—in this case, a rather subjective laboratory assistant. Today this color is interpreted by a technician in physical terms of wavelength and light intensity. Modern instrumental analysis requires a greater knowledge of physics and mathematics in order to evaluate chemical methods properly. Therefore, these subjects are discussed in relation to quantitative chemical problems. Because of the importance of analytical instrumentation to the technician, detailed instruction is given in the operation and bench maintenance of a typical spectrophotometer. The final section of this chapter deals with flame photometry. As you will learn, this principle of chemical analysis is different from absorption spectrophotometry but similar in the instrumentation used for quantitation.

11. Colorimetry

11-1. To understand the basic principles of colorimetry and spectrophotometry you must be familiar with the basic physics of light. With this knowledge you will understand the theoretical basis of photoelectric laboratory instrumentation which you use many times every day. Careful study of this section will enable you to relate what you learn about the physics of light to colorimetry and spectrophotometry as it is known today, and as it will develop in the future.

11-2. **Physics of Light.** An understanding of color measurement is necessarily based upon a knowledge of the properties of light. Most of us have seen the classical picture of Sir Isaac Newton splitting rays of light into their component colors with a prism. Conversely, the bands of color, or *spectrum,* when recombined form ordinary white light. A prism is a glass or quartz wedge which causes the light passing through it to undergo a change in velocity. The velocity of light through solids varies with the wavelength. This results in unequal bending of light rays, as their wavelengths are different for various colors. Newton was able to demonstrate this in the middle of the 17th century. Today much more is known about the properties of the spectrum, of which visible light is one relatively narrow part. Figure 11 shows the relationship of visible light to the other known wavelengths of light energy. The concept of color is physical, in that it depends upon the wavelength. Color as seen by the human eye is psychophysical, in that color is a response of the brain. Luminous energy, or light, is explained as a series of expanding wave fronts. This concept of waves was first proposed in 1800 by Thomas Young, an English scientist. The fact that one light energy can interfere with another light energy substantiates the theory that light is composed of waves of various energies or wavelengths. A wavelength is measured in terms of distance between two maximum points or two minimum points, as shown in figure 12. Values for bands of the visible spectrum are shown in table 6.

11-3. **Basis of Colorimetry.** In attempting to relate concentration of a solution to color, you will use Beer's law. This law, combined with Lambert's law, states that optical density of a colored solution is directly proportional to the number of colored molecules or ions in the path of light. In other words, the optical density is directly proportional to the concentration of the substance being measured. The substance being measured is presumably responsible for the color as a result of the chemical principle of the particular test. It is obvious that by varying the depth

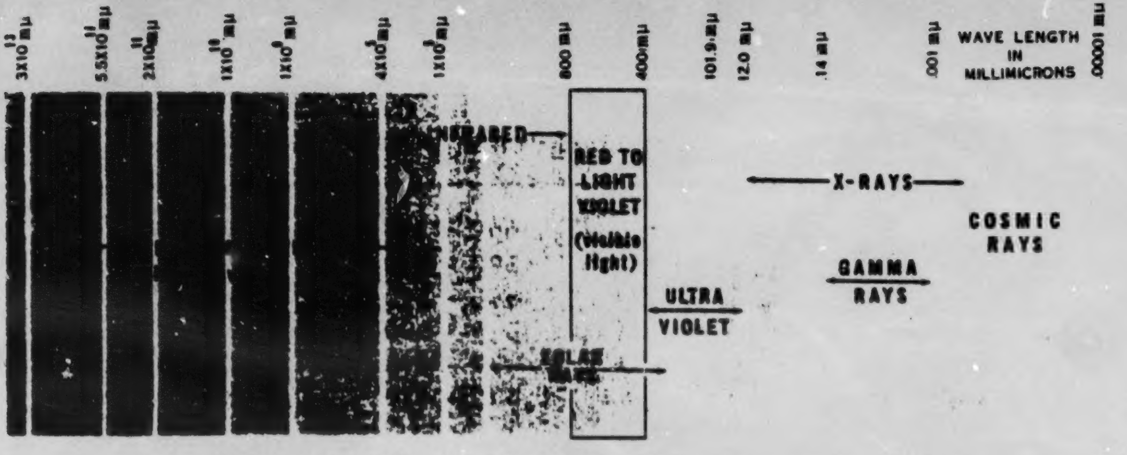

Figure 11. Position of visible spectrum with reference to other wavelengths of energy.

of the solution through which light passes, the intensity of color is also proportionally changed.

11-4. In visual colorimetry, the analyst compares the apparent color of an unknown solution with a *standard*, or solution of known concentration. For instance, assume a standard 1 percent solution of a colored substance such as dye is viewed in a tube or chamber 2 mm. deep. You find that it appears to have the same color density as a solution of the same substance in a chamber 1 mm. deep. The second solution (unknown) would, therefore, be valued at twice the concentration of the first, or 2 percent. The Beer-Lambert law is applied in this manner in Duboscq colorimeters. Other colorimeters sometimes provide a prepared visual standard in which the depth of the solution is not varied. The unknown is merely compared with a chart or color code. In a third type of colorimeter, either the unknown solution or the standard is diulted until colors match, and the dilution factor is applied as a means of calculating concentration. Most laboratories have adopted spectrophotometric analysis for routine use; however, the colorimeter is still used in specialized situations and in field laboratories.

11-5. **Basis of Spectrophotometry.** Photometry today usually refers to the electrical measurement of transmitted light rather than to visual comparison. The advantages in using a photoelectric cell instead of subjective interpretation of color intensity add significantly to the precision and accuracy of a determination. Further, photometry can use areas of the spectrum which are invisible to the human eye. A spectrophotometer is also able to "read out" colors which are invisible to the human eye. This is done by means of a reagent blank. A blank solution is placed in the light path between the light sources and a photocell, with the instrument set at 100 percent transmission. This, of course, does not mean that all of the light entering the solution strikes the photocell. It means only that the light which reaches the photocell is considered as 100 percent. Then, any decrease in transmission is a result of the colored unknown solution in the light path. This is true because a blank contains none of the color caused by the chemical reaction.

11-6. The use of a beam of light that shows maximum absorption for color developed by the test with minimum absorption for interfering colors provides the best index in determining the concentration of an unknown. As we stated earlier, components of the spectrum vary in wavelength and are selectively absorbed or transmitted

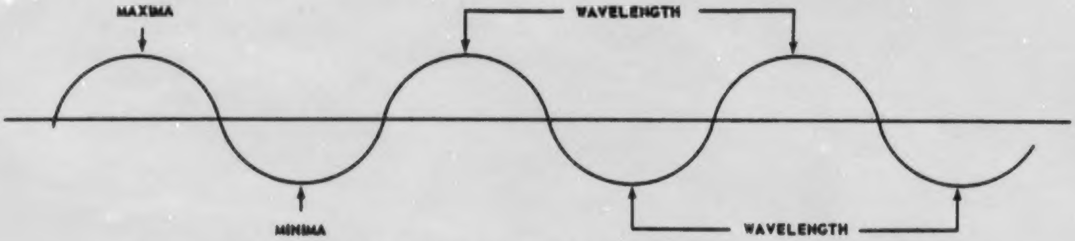

Figure 12. Diagrammatic representation of light waves, showing principle of wavelength measurement.

TABLE 6

COMPONENT WAVELENGTHS OF VISIBLE SPECTRUM

Color	Wavelength Range in Millimicrons
Red	650 - 760
Orange	590 - 650
Yellow	560 - 590
Green	490 - 560
Blue	430 - 490
Violet	380 - 430

by a solution according to the color of the solution. A red solution appears red because it reflects or transmits maximum light in the red (650 to 700 $m\mu$) area of the spectrum. Other wavelengths are transmitted to a very limited degree. From this it would appear that an instrument which uses a region of the spectrum having a color complementary to that of maximum transmission would measure maximum absorption. This is indeed true, and for this reason spectrophotometers are equipped with wavelength selectors called monochromators. The most common monochromators employ prisms, diffraction gratings, and interference filters. In using a spectrophotometer, you should select the wavelength which will achieve maximum absorption, allowing for interference bands. You can then apply Beer's law which, you may recall, states that optical density of a colored solution is directly proportional to the concentration. The light which passes through does not show a linear relationship with the concentration however. We shall describe mathematical application of this in greater detail in the following section.

11-7. **Recent Advances in Photoelectric Instruments.** Some of the latest models of spectrophotometers produce results which are recorded on a digital counter, tape, or punched card. These instruments are expensive, with one well-known model selling for $2,500. In 1963, a fully automatic commercial spectrophotometer was introduced which included a turntable that automatically feeds 60 samples into the instrument in 1 hour. Many later improvements have been made.

11-8. Spectrophotometers for infrared analyses have been available in clinical laboratories since 1950. Infrared spectrophotometers can achieve greater specificity in identification and resolution, although infrared methods are not as sensitive as ultraviolet techniques. In addition, water absorbs infrared radiation, which means that mixtures to be analyzed should be essentially free of water. Infrared spectrophotometers are particularly useful in the analysis of blood steroids and urinary calculi.

11-9. For highly sensitive studies, clinical laboratories have used fluorimeters which can detect $\frac{1}{10}$ of a part of a certain substances per billion parts. Fluorimeters measure radiant energy caused by photoluminescence. That is, some compounds emit energy after they have absorbed characteristic radiant energy. Substances measured with fluorimeters include vitamins, hormones, trace elements, and certain drugs. Fluorimeters are not necessarily new in the clinical laboratory, but they are achieving wider application.

12. Calculations and Curves

12-1. Most errors in clinical chemistry are made in the area of calculations and curves. Many of these errors involve simple arithmetic. Obviously, unless you calculate a result correctly all of your diligent work is wasted. If you are one of the few technicians who has a phobia about math, regardless of past experience, you can achieve proficiency by a positive "can do" approach to the subject. As a laboratory technician, you are most articulate when you express yourself mathematically. In this section you will study spectrophotometric calculations and the major types of curves.

12-2. **Spectrophotometer Calculations.** Visual colorimetry is unlike spectrophotometry in that in the latter there is no need to change the depth of solution through which the selected beam of light passes. The concentration of the unknown is related to the concentration of the standard, based on Beer's law, which is stated mathematically as follows:

$$\frac{OD_u}{OD_s} \times C_s = C_u$$

where

OD_u = optical density of the unknown
OD_s = optical density of the standard
C_s = concentration of the standard
C_u = concentration of the unknown

The answer will always be in the same terms as concentration of the standard. For example, if a standard is expressed in mg-%, the value of the unknown will be in mg-%. There are certain limitations to this whole concept, however. Beer's law is not valid at extremely high concentrations. How do you know at which concentration deviation will take place? This must be determined for each procedure when the procedure is established. Reaction mixtures exceeding the linear range in concentration may rarely be diluted if you expect accuracy. In most cases, it is necessary to repeat the entire procedure using a dilution of the original specimen; this procedure is recommended.

12-3. Another limitation to Beer's law is that it applies only to clear solutions. Turbidity changes the linear relationship between color and concentration because light rays are scattered by suspended particles (Tyndall effect). Concentrations of turbid solutions are measured with special devices or they may be estimated with a spectrophotometer. But, strictly speaking, Beer's law is not followed for turbid solutions. If turbidity happens to develop along with a color, the test is performed again or the solution must be cleared by filtration or centrifugation. Returning to Beer's law, and assuming that it applies to the particular test, you may express C_s in milligrams (or other units) of standard present. As is the case with any other statement of concentration, C_s is an expression of the amount of solute present. C_s equals the amount of standard in 1 ml. times the number of milliliters used in the procedure. As stated earlier, C_s may be given in mg-%, and is arrived at as follows:

$$\text{Ml. of std used} \times \text{mg. per ml. in std} \times 100 = \text{mg-\%}$$

You would encounter a complication only if, in actually performing the test, you used a volume of unknown different from the volume of standard. You would then need to correct for the difference in volume between the unknown and the standard with a "*V* factor." Mathematically, this factor is as follows:

$$V = \frac{\text{ml. serum or actual specimen} \times \text{ml. diluted spec. used}}{\text{total volume of diluted specimen}}$$

For example, if 2 ml. of serum are used in preparing 20 ml. of a protein-free filtrate (PFF), and 1 ml. of the PFF is used, the *V* factor is:

$$V = \frac{(2)\ (1)}{20} = 0.1$$

This *V* factor is placed in the original spectrophotometer formula as follows:

$$C_u = \frac{OD_u}{OD_s} \times C_s \times \frac{100}{V}$$

Remember, the *V* factor is necessary to take into consideration any difference in volume between the amount of the standard and the amount of unknown specimen used in the procedure.

12-4. One final factor enters into the spectrophotometer formula. It is quite simple and seldom applies. If the final volume of the unknown solution differs from the final volume of the standard, a dilution factor is supplied. The formula then becomes:

$$C_u = \frac{OD_u}{OD_s} \times C_s \times \frac{100}{V} \times \frac{\text{final volume of unknown}}{\text{final volume of standard}}$$

Usually the unknown is treated exactly like the standard and there is no need to correct for different final volumes. But if the unknown should be diluted with an equal volume, for example, multiplication of C_u by 2 would correct the equation.

12-5. Problem Situation No. 1: In a BUN procedure, the standard consists of 0.05 mg. nitrogen per milliliter, and 3 ml. are used in the test. Calculate the C_s factor.

12-6. Problem Situation No. 2: The BUN method you are using requires 5 ml. of a PFF prepared as follows: 16 ml. of *N*/12 sulfuric acid, 2 ml. of serum, and 2 ml. of 10 percent sodium tungstate. Calculate the *V* factor.

12-7. Problem Situation No. 3: In performing the BUN procedure described in the preceding two paragraphs, you discover that different final volumes of the standard and unknown are obtained. The final color development mixture of the standard is 10 ml. and the final color development mixture of the unknown is 5 ml. Calculate the dilution factor which must be considered.

12-8. Problem Situation No. 4: The percent transmittance (percent *T*) of the BUN standard is 60.25 percent ($OD = 0.220$). The percent *T* of the unknown is 51.5 percent ($OD = 0.2888$). Using this data and all of the factors calculated in paragraphs 12-4, 12-5, and 12-6, determine the concentration of the unknown specimen in milligrams per 100 ml. to three significant figures.

12-9. Solution to Problem Situation No. 1:

$$C_s = 0.05 \text{ mg./ml.} \times 3 \text{ ml.}$$
$$C_s = 0.15 \text{ mg.}$$

12-10. Solution to Problem Situation No. 2:

$$V = \frac{2 \times 5}{20}$$
$$V = 0.5 \text{ ml.}$$

12-11. Solution to Problem Situation No. 3:

$$\text{Dilution factor } (F) = \frac{\text{final volume of unknown}}{\text{final volume of standard}}$$
$$F = \frac{5}{10}$$
$$F = 0.5$$

12-12. Solution to Problem Situation No. 4:

$$C_u = \frac{OD_u}{OD_s} \times C_s \times \frac{100}{V} \times F$$
$$C_u = \frac{0.288}{0.220} \times 0.15 \times \frac{100}{0.5} \times 0.5$$
$$C_u = 19.6 \text{ mg. per 100 ml.}$$

12-13. **Calibration Curves.** If the dilution factor, depth of cuvette, and chemical aspects of a procedure (e.g., incubation time) are kept constant, the standard solution should theoretically read the same from day to day. In reality, standards are seldom that reliable for all procedures. But for the purposes of introducing the concept of calibration curves, we shall assume that the standard of a particular concentration consistently yields a particular optical density reading. A second standard of ½ the concentration should read at ½ the optical density, a third standard of ¼ the concentration of the first should be read at ¼ the optical density. As a result, if a series of such standards is charted on graph paper, the results will be a straight line for any test procedure which follows Beer's law, as shown in figure 13. Specimens treated in the same way as the standard could be read from this same curve. All curves used in the clinical laboratory are not necessarily straight line segments. If enough points are plotted, a nonlinear curve, such as an SGOT curve, is quite useful. This curve is shown in figure 14. In fact, you will find that enzyme curves seldom follow a straight line.

12-14. A curve in which concentration is plotted versus absorption is called a concentration absorbance (C-A) curve. The straight line (linear) curve mentioned in the previous paragraph and illustrated in figure 13 is a C-A curve. It is used in lieu of calculations involving the spectrophotometer formula, but it should be used with caution, because what holds true under one set of circumstances may not hold true under another. In the case of a curve which does not follow Beer's law as shown in figure 14, the mathematics involved is too complicated to achieve a reportable result effectively without the aid of a graph. Thus, we have at least two reasons for using C-A curves. They save time and simplify calculations. Used properly, C-A curves are of great value to the clinical laboratory. The spectrophotometer scale is usually calibrated in percent T as well as optical density. It is generally easier to read percent transmittance, but as stated previously the relationship between concentration and percent transmittance is not a direct proportion as it is between concentration and optical density. This is because optical density equals the expression $-\log T$ or $\log \frac{1}{T}$. In this respect several factors should be noted.

a. Optical density is synonymous with absorbancy. In the literature of this field, various designations are assigned to this value, such as *OD*, *D*, A_s, a_s, *A*, *a*, or *E* (extinction).

b. Optical density (or absorbancy) is logarithmically proportional to transmittance or percent transmittance.

c. *T* in this formula refers to transmittance and *not* to percent transmittance (percent T). Percent transmittance is $100 \times T$ (percent $T = 100 \times T$). This quantity may be referred to as *T*, T_s, or *I*. Thus, it is possible to achieve a linear graph if percent T is plotted on semilog graph paper rather than on ordinary graph paper (C-T curve). Such a graph is just as useful as any other type except that semilog graph paper is harder to read. It is important to observe that labeling of the vertical or y-axis usually begins at ten. The midpoint between the lower left-hand corner and 20 percent T is not 10, as can be seen by a study of figure 15. Errors are frequently made in labeling the y-axis.

12-15. **Spectral Absorbance (S-A) and Spectral Transmittance (S-T) Curves.** A spectral absorbance curve enables you to obtain the wavelength at which maximum absorption takes place with a given solution. You have just learned that maximum benefit is derived from Beer's law if a wavelength of maximum absorption is used. In a sense, this is a trial-and-error sort of curve in which the optical density (O.D.) is recorded as the wavelength is changed in small increments. The wavelength at which maximum optical density is observed is then chosen as the wavelength for that particular determination, providing no further correction for interference because of natural or extraneous color is required. If percent transmittance is plotted versus wavelength, instead of O.D. versus wavelength, an inverted curve results, but all of the principles are the same. Such a curve is called a spectral transmittance curve. It is not necessary to plot the S-T curve on semilog paper because no linear relationship is shown. The various points are plotted as shown in figure 16. The purpose of the plot is limited to showing the lowest point or points of the curve. This is particularly impor-

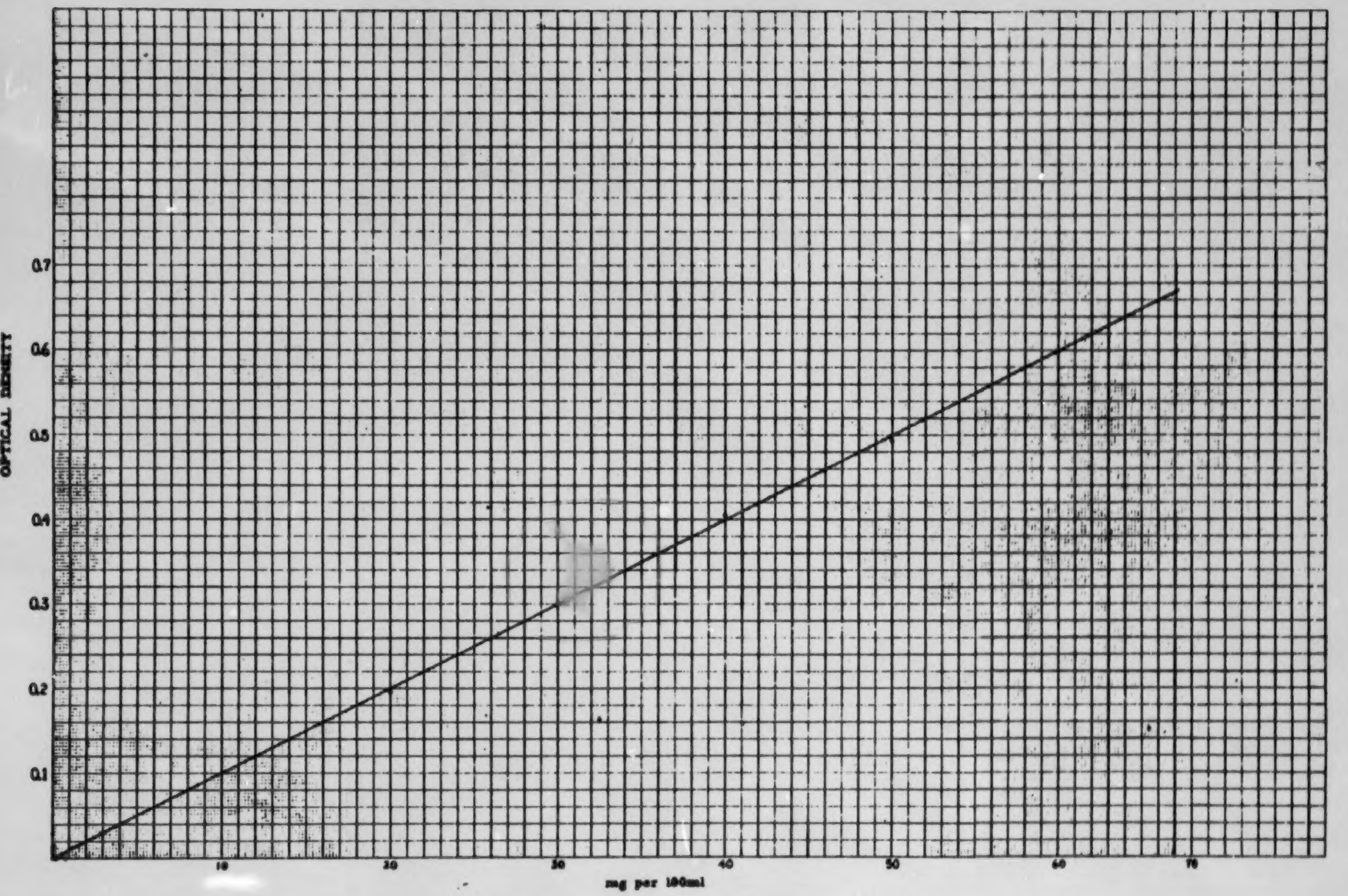

Figure 13. Concentration-absorbance curve.

tant if absorption is in the infrared or ultraviolet range, although S-T curves are valuable at various wavelengths.

12-16. **Operation of Spectrophotometer.** Certain problems arise which cause significant errors with spectrophotometric determinations. First, the electrical supply must not show excessive voltage or frequency fluctuations. The best way to avoid "drift" resulting from these line fluctuations is to supply power from a wet battery equipped with a trickle charger. Electronic power supplies are available which solve the problem of limited line power fluctuations. Second, cuvettes must be properly matched. You are familiar with the classification of cuvettes and undoubtedly realize that you should not mix categories of cuvettes. There is no guarantee that individual cuvettes of the same category will match. If two cuvettes vary optically by a few percent T, the effect on a determination can be quite significant. Since you will probably not choose to read several determinations in one cuvette, the practice of matching cuvettes would be helpful in reducing this error. Care of cuvettes is as important as care of the optical components of an instrument. Finally, as with any instrument, a spectrophotometer should not be used with blind faith. Optical components may become dusty, lamps age, and any number of electrical or mechanical defects can affect the accuracy of your determinations. Any result, no matter how carefully achieved is only as good as the instrument that is used to attain it.

12-17. We shall discuss quality control in clinical chemistry in Chapter 6, but quality control in spectrophotometry is a necessary part of any discussion of spectrophotometry. Since the most familiar instrument in military laboratories is the Coleman, Jr. Spectrophotometer, we shall discuss it in detail in the following paragraphs.

12-18. *Exciter lamp check.* The exciter lamp must be checked before wavelength calibration and before the spectrophotometer is calibrated for a specific determination. The wavelength calibration alone does not adequately fulfill various requirements of a light source. Turn the instrument on and allow 5 minutes for it to warmup. Then, to check the photocell, exciter lamp, and certain other aspects of the electronics system, set the wavelength at 400 mμ with the cuvette well empty and the galvanometer controls at their counterclockwise limits. Cover the cuvette well and turn the galvanometer controls clockwise. The galvanometer hairline should go off scale to the right past 100 percent T. If it does not do so, the reason may be failure in the exciter lamp, photosensitive cell, wiring, or power supply. If the calibration is not within these limits, turn the instrument off, *unplug it from its power source* at the connector plug (H in fig. 17), and turn the instrument over gently. Remove the bottom metal cover, as shown in figure 17. Locate the exciter lamp (A) and galvanometer lamp (C). Remove the exciter lamp as follows:

Step 1. Disconnect the lamp by loosening it from the terminals (D).

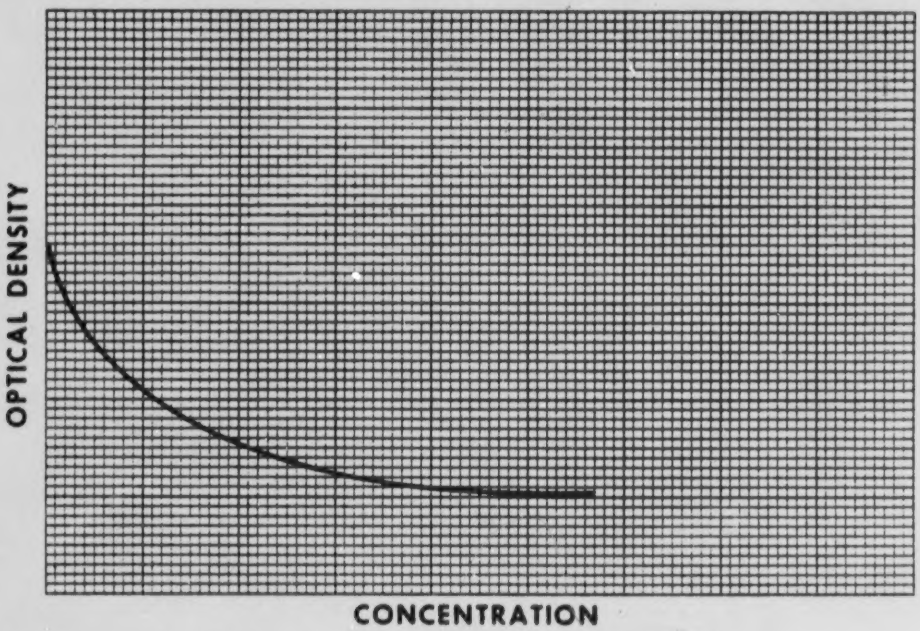

Figure 14. Nonlinear curve as used in enzyme studies.

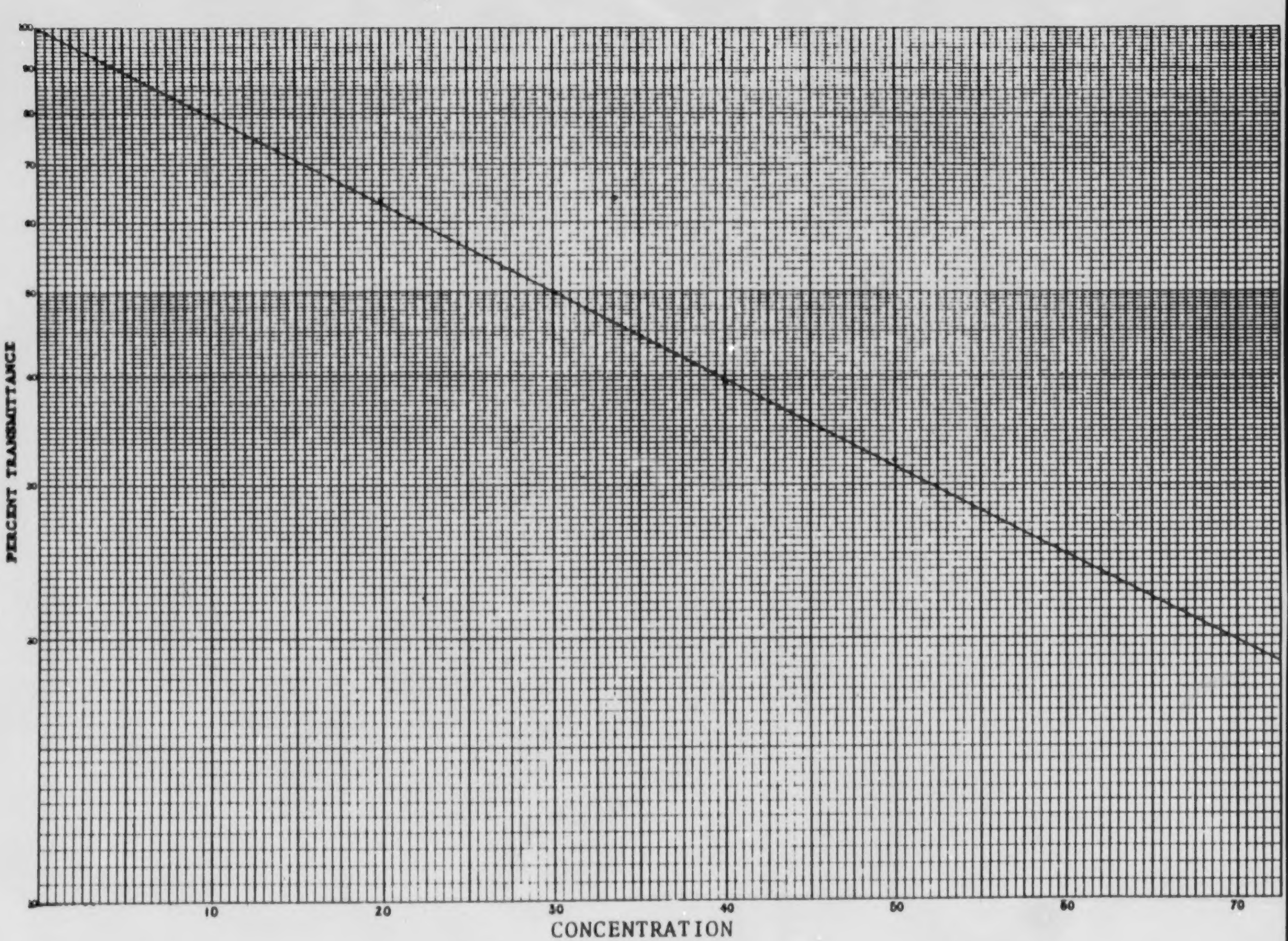

Figure 15. Concentration transmittance curve.

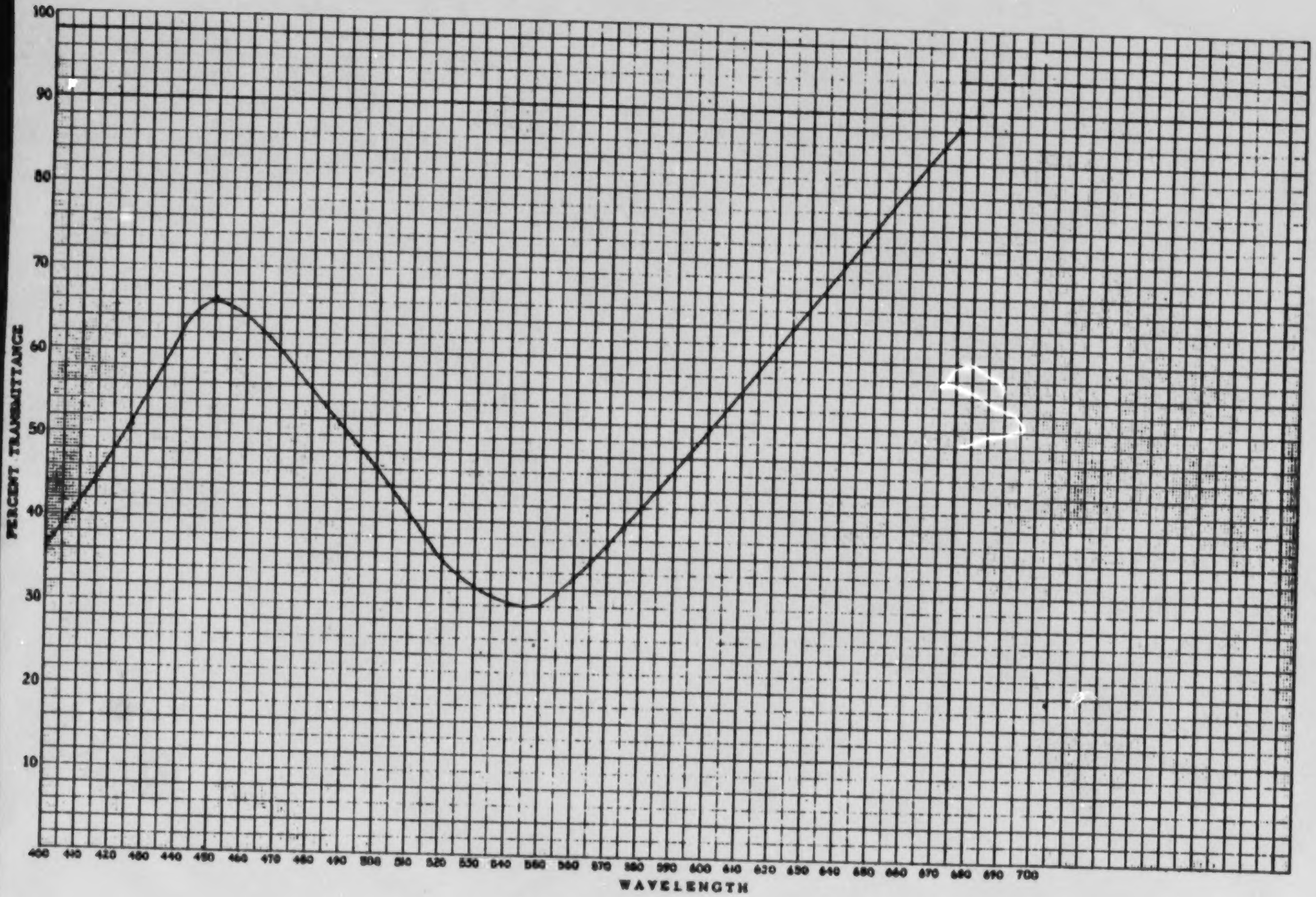

Figure 16. Spectral absorbance curve.

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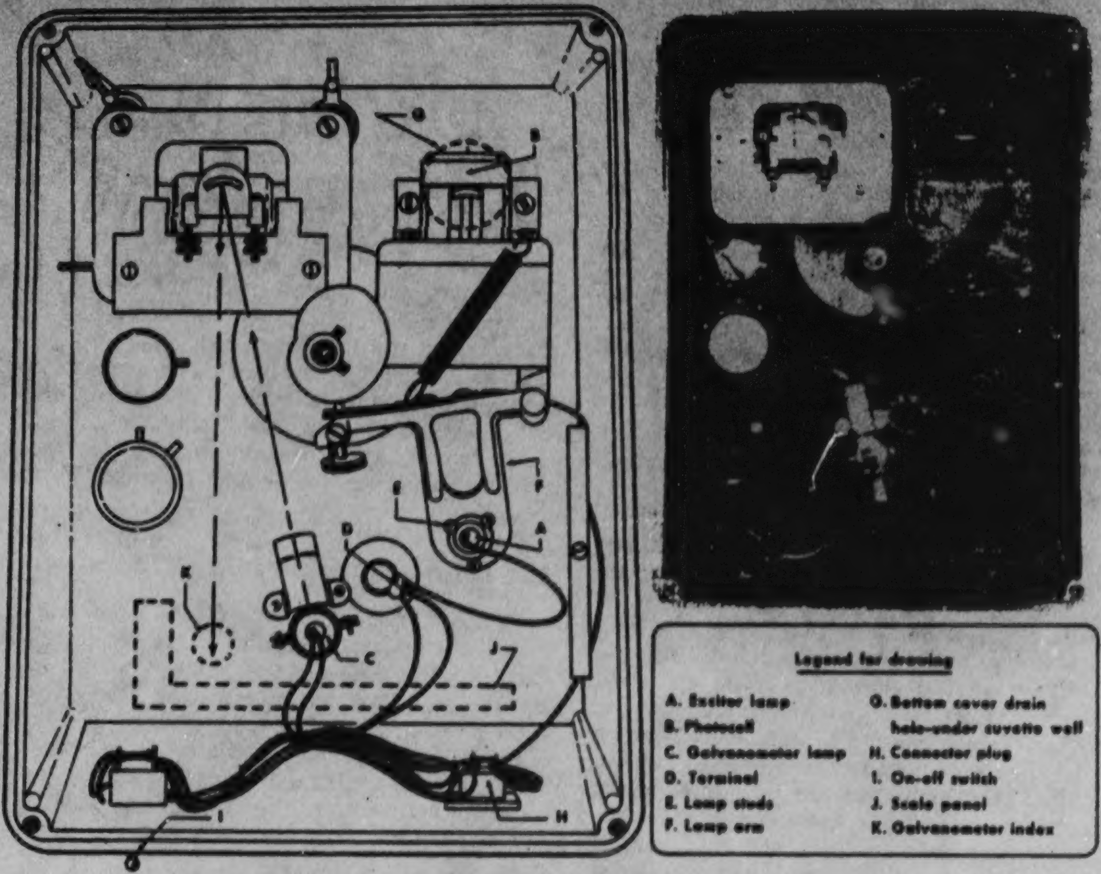

Legend for drawing

A. Exciter lamp
B. Photocell
C. Galvanometer lamp
D. Terminal
E. Lamp studs
F. Lamp arm
G. Bottom cover drain hole-under cuvette well
H. Connector plug
I. On-off switch
J. Scale panel
K. Galvanometer index

Figure 17. Bottom view of the Coleman, Jr. spectrophotometer.

Step 2. Place a 50-cent piece or another metal disk approximately the same size on the three spring lamp studs (E).

Step 3. Place the thumb of your left hand on the coin with your index and second fingers under the lamp arm (F). Use gentle pressure to press the coin down, releasing the lamp studs while restraining the lamp arm.

Step 4. With your right hand, turn the lamp clockwise as viewed from the bottom, to release the lamp. (Note that the filament is toward the lens system.)

Step 5. To replace the lamp, reverse this procedure using a piece of lens paper to hold the lamp so that fingerprints will not be made on the lamp surface.

12-19. When the exciter lamp is removed, check it for darkening and frosting and clean it with a damp cotton ball. Replace the lamp if it does not check as being serviceable in accordance with paragraphs 12-20, 12-21, and 12-22. Always clean a new exciter lamp before installing it and be careful not to dirty it. Also, examine all lenses and slits for cleanliness. Be very careful when cleaning lens surfaces, using a clean piece of lens paper dampened with alcohol for each lens. Check the cuvette well slit and phototube for cleanliness. *Do not force* any of the mechanism to reach these surfaces. Use a dampened cloth to dust inside surfaces of the case and to clean the scale panel (J in fig. 17). After cleaning the exciter lamp and lens surfaces, repeat the 400-mμ check as given above. If this still does not check, the lamp should be replaced with a new exciter lamp. Then wavelength and 400-mμ check should be repeated. If the check is still not satisfactory, the wiring and power supply should be inspected. The photosensitive tube may be weak or defective if the wiring and power supply are in order.

12-20. *Lamp darkening check.* It should be a daily routine for you to run a lamp darkening check along with an exciter lamp check. First, run a wavelength calibration, then change wavelength from 610 mμ to 590 mμ and reset 100 percent T, using the galvanometer controls. Now insert a calibration filter again and take a reading in percent T. Repeat this procedure to insure the

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reading. (Note the particular value for this exciter lamp, when clean and new, in this spectrophotometer.) It is advisable to write this value on a label attached to the spectrophotometer. In later routine readings, this value for percent T must not vary more than ±1.5 percent T from the original reading of the new exciter lamp when initially installed. If the value exceeds this tolerance, remove the exciter lamp and inspect it for darkening or frosting. If the exciter lamp can be cleaned, recheck at 400 mμ and run a wavelength calibration. A permanently darkened lamp sould be replaced and all quality control checks rerun.

12-21. *Calibration.* A didymium calibrating filter is supplied with the spectrophotometer. The wavelength to be used and percent T reading are printed on the plastic adapter, i.e., $\lambda =$ 610 mμ; T = 0.46. Always clean the filter with alcohol and cotton before each use. Turn the instrument on and allow it to warm up for at least 5 minutes. Set the wavelength dial to 610 mμ. Zero is aligned by turning the cuvette holder crosswise in the cuvette well to cut out all light. Turn the coarse and fine galvanometer dials to their counterclockwise limits. Move the plastic galvanometer scale to place the hairline on zero. If the scale movement is not sufficient to set zero, very gently move the galvanometer adjustment lever to the right or left. Then, move the scale panel again for fine adjustment. Set 100 percent T by first removing the cuvette holder from the cuvette well and covering the well. Turn the fine galvanometer dial one turn clockwise, and turn the coarse galvanometer dial to move the hairline (galvanometer index, fig. 17) within ±5 percent T of 100 percent. Use the fine galvanometer for fine adjustment to 100 percent T. Allow the galvanometer to settle for a few minutes and observe its stability. If the hairline wanders or is very erratic, this is probably a result of line voltage fluctuations or a faulty power supply. A medical equipment repair technician should check both thoroughly. A constant voltage transformer will not always stabilize line voltage fluctuations. An electronic power supply or battery source may be necessary to overcome this instrument error. The power source must be reliable before continuing with any calibration or use of this instrument. After 0 and 100 percent T are set, align the calibrating filter in the cuvette well. This reading must be within ±2 percent T of the inscribed percent T reading for good calibration. If the reading is not within these limits, turn the instrument over, remove the cover, and locate the calibrating screw (F in fig. 17) on the lamp arm. If percent T value is *less* than percent T inscribed on the filter, turn the calibrating screw two clicks clockwise for each percent T difference; or turn it counterclockwise two clicks for each percent T more than the calibration value. Check the reading again after each adjustment. Remember to unplug the instrument while working inside of it. Also remember that a 5-minute warmup is required before making a reading. After changing the calibrating screw and verifying the calibration, run an exciter lamp check before you close the cover.

12-22. Figure 18 may be used as a checklist in making the exciter lamp check, the lamp darkening check, and wavelength calibrations. You should have a copy of this or a similar checklist available whenever you use a spectrophotometer.

13. Flame Photometry

13-1. It is evident that you will be called upon to perform certain procedures on an emergency basis. In these instances, you will not be able to hesitate or debate the problems at hand. The flame photometer is often required in performing emergency and routine procedures. It is important that you feel confident under adverse, as well as normal, working conditions. Consequently, you have a real need to study the principles of flame photometry outlined in this chapter.

13-2. **Principles of Flame Photometry.** The serum, urine, or other specimen is vaporized by aspiration into a flame to produce certain colors of light. Color which is characteristic of the light emitted by the constituent being measured is passed through a filter to a photoelectric cell where its intensity is measured. By comparing the intensity of emitted light with that of a standard, concentrations of metallic ions can be determined. This principle is commonly applied to the measurement of sodium and potassium, as well as calcium. Some clinical laboratories may also perform serum magnesium levels, but the significance of magnesium has not yet been adequately developed as a diagnostic procedure to warrant performance in all clinical laboratories. Further, the concentration of magnesium may be too low to permit accurate direct measurement. A convenient technique when measuring metallic ions in low concentration is to add a known number of milliequivalent weights of the ion to the unknown specimen. It must be kept in mind with all flame photometric determinations that you are working with very low concentrations. A result of 4 mEq/L. of potassium, for example, is equivalent to 0.156 mg./ml. The slightest contamination can drastically change this result; a few tiny flakes of soap powder could easily change the

Exciter Lamp Check

a.	Passes 100% T.	Proceed with lamp dark, check.
b.	Does not pass 100% T.	Check bulb for frosting or darkening - clean and recheck.
(1)	Permanently darkened.	Replace exciter lamp.
(2)	Cleaned and does not recheck.	Replace exciter lamp.
(3)	New lamp does not check.	Check wiring, power supply, and phototube.
c.	Recheck weekly.	

Lamp Darkening Check

a.	Record % T new lamp.	
b.	Checks within ± 1. 5% T.	Run wave-length calibration.
c.	Variation greater than 1. 5% T from original value.	Check exciter lamp for frosting or darkening.
(a)	Cleaned.	Recheck.
(b)	Cleaned and does not check.	Replace exciter lamp.
(c)	Permanently darkened.	Replace exciter lamp.
(d)	New lamp.	Run exciter lamp check and record % T new lamp.
d.	Recheck daily.	

Wave-Length Calibration

a.	Zero set.	
b.	100% T set.	
c.	Filter reading.	
(a)	Reads within ± 2% T.	Spectral quality O. K.
(b)	Reads outside ± 2% T.	Run exciter lamp + darkening check.
(c)	Exciter lamp and darkening check O. K.	Change calibration.
(d)	Recheck filter reading.	

Figure 18. Summary of procedure to minimize error with the Coleman, Jr. spectrophotometer.

value of an electrolyte result by 100 percent. For this reason, it is advisable to clean all glassware with nonionic detergents which are compounds that contain no metallic ions. They are available through commercial sources, and reduce the possibility of adding sodium or potassium ions by contamination. Glassware must still be thoroughly rinsed in deionized water because even nonionic detergents interfer with some studies, especially blood alcohol analyses. Of course, the water must be pure and you should never use your finger to stopper a volumetric flask when you are diluting specimens.

13-3. Always keep the parts of a flame photometer clean and free from atmospheric contamination. Perhaps you have observed how a Bunsen burner will glow brightly when a detergent or dry bacteriological media are dispensed in the vicinity. The same effect is produced with a photometer flame in a contaminated atmosphere, and this is a source of error. For this reason, smoking should be prohibited near an operating flame photometer. If the flame cone is surrounded by a wire, this screen must not be worn or irregular. A torn screen will cause aberration of the flame. Operating details for an instrument are always available in the operation manual provided by the manufacturer of the instrument. Within reason, instructions of the manufacturer generally take precedence over information in textbooks. One technique which seeks to eliminate variables such as conditions of ion excitation is called the method of *internal standards.* This method amounts to measuring the unknown emission line with respect to that of a known standard spectral line. The use of internal standards becomes quite involved and has its limitations. However, it does eliminate some of the disturbing influences on flame photometry. The use of these standards is not to be confused with the use of ordinary standards for determining unknowns.

13-4. **Components of Flame Photometers.** The flame must be supplied with fuel of constant pressure by means of *pressure regulators.* The oxygen tank is usually equipped with a needle valve for this purpose. This valve should be handled with reasonable care. A second component of the flame photometer is an *atomizer,* which aspirates the specimen into the flame. Most atomizers consume from 1 to 2 ml. of sample per minute. A third component is the *burner* itself. The flame should burn evenly and not strike back or sputter. Particles of dirt are most often responsible for uneven burning, assuming that the fuel supply is constant. The fourth essential component of a flame photometer is the *optical system.* Some models do not use filters to isolate the characteristic band of light; instead, they employ a monochromator system and a more sensitive photodetector. These instruments are generally more expensive than devices with glass filters, but they are more selective and can be used with metallic ions for which filters are not suitable. A fifth component of the instrument is a *photosensitive detector;* its purpose is to measure the intensity of the emitted band characteristic of the constituent being measured. This may consist of a simple phototube or a photomultiplier tube. For research use, particularly in work with lithium, cesium, and rubidium, the photomultiplier type is preferred. The last component is the *indicating device.* Most often this is a galvanometer with a light indicator.

13-5. **Mechanism of Spectrum Production.** Upon being atomized into a flame, water evaporates from the sample. Solid particles which are left then burn or decompose. Both vaporization and ionization of the elements may occur. The heat energy of the flame "excites" the atoms or molecules. This phenomenon is actually one of raising the electrons to higher energy levels. This energy is then given off as radiant energy when the electrons return to their former energy levels. For molecules, there are additional energy considerations which must be taken into account in explaining the spectrum. Finally, there is a spectrum resulting from the flame fuel itself and the fuel-oxygen ratio. The fuel is usually selected in accordance with the analysis to be performed. The use of oxygen significantly increases the temperature of the flame. For example, the maximum temperature for illuminating gas in air is approximately 1700° C., whereas in oxygen it is 2700° C. Appropriate care must be taken in the use and storage of oxygen because it has the property of supporting combustion although it does not burn itself.

CHAPTER 5

Specimen Collection and Preparation

THE TESTS YOU will perform in the laboratory will be of absolutely no value if you do not have adequate or suitable specimens. In most cases, it is your responsibility to secure the specimen, or at least to provide instructions for collecting it. In dealing with patients, you should be thoughtful and professional in your approach; however, with specimens an objective impersonal approach is called for. This approach enables you to serve the needs of the patient best by providing reliable laboratory data useful to the clinician.

2. The type of anticoagulant you use, the type and quantity of specimens to collect, and the way to handle the specimens are all points of technical importance. For example, a specimen for blood glucose which has been allowed to remain at room temperature may deteriorate from an abnormally high value to a normal or an equivocal value within hours. An erroneous result is of far less value to the physician and is far more harmful to the patient than no result at all. To be proficient in this career field, you must have the facts as well as the will and ability to use them. You do not work efficiently from habit or administrative discipline alone. With a thorough working knowledge of specimen requirements, you will be able to fulfill a fundamental requirement essential to providing reliable laboratory data. Patients should not be inconvenienced without good cause. As a competent technician, you should keep to a minimum reports of "q.n.s." (quantity not sufficient), "specimen broken," etc. Of course, there are times when another specimen must be obtained. However, a technician who knows how to collect and handle specimens properly can usually perform the tests required with the least effort and trauma to the patient. Whenever a problem arises concerning specimens, technical and medical requirements should be considered before a specimen is deemed unsatisfactory. Your own ingenuity and coordination with the requesting physician will often alleviate difficulties such as specimen inadequacy without further involvement with the patient.

3. At times you must do more than you are requested to do. Your alertness in detecting conspicuous abnormalities, e.g., jaundiced serum, hematuria, and lipemia, can be an invaluable diagnostic aid. The material presented here should supplement your previous training in achieving this end. It is also appropriate to remind you that you are part of a service, the Medical Service of the U.S. Air Force. Therefore, you function for the benefit of others, and it is the patient who should be uppermost in your mind. You can perform no higher service than applying the scientific skills you have mastered in a dedicated and understanding manner.

14. Collection and Preservation of Specimens

14-1. Before you begin any test, it is necessary to procure a suitable specimen. What is your understanding of the term "suitable?" Read the following paragraphs and evaluate your concept of a suitable specimen.

14-2. **Type of Specimen.** The procedures which a particular laboratory performs generally dictate the types of specimens required. If one laboratory performs serum frog tests and another performs urinary gonadotropin tests, the specimens are obviously different, though both are tests for pregnancy. It is well to know the general acceptability of substituting one type of specimen, such as serum, for another type, such as plasma. It may become a matter of habit to perform a procedure such as the urea nitrogen (BUN) test on whole blood. If serum is drawn in error, it may become routine for the patient to return for another venipuncture, when actually serum is acceptable as a specimen. It must be emphasized again that specimen requirements vary with the procedure followed by a particular laboratory. Ordinarily, serum differs from plasma only in that plasma contains fibrinogen and possibly a few factors which disappear from serum during clot formation. Except for a slight loss of CO_2 from serum as a result of clot formation, the difference between serum and plasma is negligible for routine use. (An exception is the prothrombin determination, which requires fibrinogen.) Whole blood is generally used for analyses which include alcohol, carbon monoxide, and glucose.

14-3. **Anticoagulants.** When using whole blood, you must be aware of which anticoagulant should *not* be used. For example, anticoagulants which contain ammonium oxalate must never be used for urea nitrogen determinations. In the Air Force Medical Service most anticoagulants are standard and are supplied in tubes which have color-coded stoppers. They include lithium oxalate, double oxalate, EDTA, and heparin. Sodium citrate may also be used, but it is not the anticoagulant of choice for most chemistry procedures, because of the water shift it produces. Heparin is an effective anticoagulant, functioning by its interference with the production and activity of thrombin. Heparin should not be used for sodium determinations because of its sodium content. There appears to be no objection to the use of EDTA except a prolongation of the prothrombin time and possible interference with urea nitrogen determinations. Oxalates function by removing calcium from the coagulation process, and are the most commonly used anticoagulants. There are technical objections to using oxalates, one difficulty is shrinkage of cells which results in a plasma dilution. This problem is largely overcome with Heller and Paul double oxalate, but ammonium oxalate and potassium oxalate are not suitable for BUN procedures, calcium, sodium, or potassium assays. Lithium oxalate cannot be used for calcium studies, but it is otherwise generally considered the best oxalate to use for clinical chemistry specimens.

14-4. **Drawing and Storing Specimens.** The widespread practice of drawing blood specimens from patients in the fasting state appears more difficult to explain from a technical standpoint than from a standpoint of administrative convenience. Laboratory personnel ordinarily use the fasting requirement in scheduling patients simply because of this administrative convenience. Clinical chemistry has not yet matured to the point where it can be stated unequivocally the extent to which intestinal absorption of food interferes with each and every type of test. Certainly the pertinence to a particular test must be considered, especially when a cloudy serum is obtained. Some difficulty has been reported with uric acid determinations, and cholesterol is reported to be elevated following a meal which is high in cholesterol. Some chromagens present in food may produce icteric serum, and postprandial glucose will lower phosphorus values. If you do not know the possible effects of eating upon a particular test, you may feel safer in requiring a fasting specimen. It is difficult to understand why some laboratory technicians instruct patients not to drink water for several hours before drawing a specimen, since it is advisable to prevent dehydration which could result from inadequate fluid intake. Neither does there seem to be any clear purpose for prohibiting smoking before drawing blood specimens. A fasting blood sugar would obviously be accomplished in the fasting state.

14-5. After a patient has complied with instructions from you, you should proceed to draw the specimen. Always avoid hemolyzing the blood. The use of wet syringes is conducive to hemolysis. However, this may be a hypothetical point if your laboratory uses vacuum tubes or disposable syringes. Vacuum tubes may cause hemolysis if the needle is withdrawn from the vein without first disengaging the vacuum tube. Blood should never be forced out of a syringe through the needle into a container, because this causes hemolysis. Excessive withdrawal pressure on the barrel of a syringe may also cause hemolysis. Prolonged use of a tourniquet can even alter certain results.

14-6. Separation of cells from the fluid portion should be as rapid as feasible to prevent shifts that occur between cells and extracellular fluid. For example, a potassium shift would elevate the serum potassium level.

14-7. The best way to store specimens is at low temperatures in the dark. Freezing serum or plasma is usually acceptable but is not usually necessary for brief periods of storage. Nearly all enzymes are stable if they are frozen, with the possible exception of phosphatases; however, freezing followed by thawing and refreezing should be avoided. Serum for a cephalin flocculation test cannot be frozen because of the denaturing effect of freezing upon protein. Light can significantly reduce bilirubin and other values, so some attention should be given to protecting specimens from light. Glucose may be stored at room temperature for a few hours if protein-free filtrate (PFF) is prepared, and if the filtrate is covered to prevent evaporation and contamination. For a longer period of time, PFFs should be kept refrigerated. In general, it should be unnecessary to keep a PFF longer than 12 hours, but if a PFF must be preserved, it can be frozen or preserved with toluene.

14-8. Sodium fluoride is an excellent preservative for blood specimens, if there is no extensive bacterial contamination. Sodium or potassium fluoride is also an anticoagulant if used in high enough concentrations, but amounts adequate for anticoagulation produce undesirable intracellular and extracellular shifts. Therefore, fluorides are best classified as preservatives and are used most frequently in conjunction with specimens for glucose analysis. Specimens which contain fluorides cannot be used for enzyme tests. The practice of saving clinical specimens for several days until they are analyzed invites error in most procedures performed in the clinical laboratory.

14-9. Area laboratories generally establish their own requirements for referral specimens. Whenever a specimen is referred to another laboratory, it should be labeled with all of the data necessary to identify it, the date drawn, and preparation details. For example, if a preservative has been added, the container should be labeled with this information. There is sometimes a problem in the stability of certain specimens which may prevent their being referred to another laboratory. If transportation requires time in excess of that which is known to cause deterioration, there is no point in shipping clinical material just to secure some type of report. It is desirable for the performing laboratory to maintain control of specimens to insure that improper handling or storage has not invalidated results. This is not always possible, and it then becomes the responsibility of the referring laboratory to handle materials with a knowledge of chemical principles and requirements of the test requested. If blood, urine, and other substances must be shipped to another laboratory, they must be shipped promptly and in good condition.

14-10. **Handling CSF in the Laboratory.** Just before the turn of the century it was discovered that spinal fluid could be examined to aid in the diagnosis and treatment of disease. Since that time it has become quite customary to include the analysis of spinal fluid in clinical workups, and the number of tests which laboratories can perform on spinal fluids is steadily increasing. However, spinal fluid is more difficult to obtain than most other specimens; and this, consequently, places more responsibilities on the laboratory as far as proper handling of specimens is concerned. It is often stressed that spinal fluid is highly dangerous material as a potential source of contagion to the laboratory technician. This popular concept is not entirely wrong, nor is it entirely correct, because it overemphasizes the dangers of handling cerebrospinal fluid (CSF). Spinal fluid is a source of infection, just as any other biological material is, and you cannot afford to be careless with spinal fluid or with any other body fluid. Perhaps a general lack of familiarity with CSF is responsible for people maximizing its danger and treating it more cautiously than they do blood or urine. Rather than stress the highly infectious nature of spinal fluid, it would be more appropriate to stress proper handling to avoid errors or loss of the sample.

14-11. The entire reservoir of CSF in the body rarely exceeds 150 ml. The physician seldom obtains more than 8 or 10 ml., and he usually obtains much less; therefore, none can be wasted. A physician usually submits spinal fluid to the laboratory in three tubes numbered consecutively as they are drawn. The tubes are numbered to distinguish the initial tube (which is most often contaminated with blood from the trauma of the spinal tap) from the second and third tubes. The second tube contains less cellular contamination and the third tube is for cellular analysis of the spinal fluid. If vials come directly from the patient's room or from surgery, they may not be properly labeled. In this case, the first thing you should do is label the tubes. It has become generally accepted that tube number 2 is for chemistry determinations. Other tubes may also be used for chemistry, if they are free of blood.

14-12. The possibility of centrifuging cells from a bloody tube and using the supernatant for chemistries is not to be excluded. But it is not usually considered acceptable to do so when the purpose is to remove a significant amount of blood from a traumatic puncture. If a tube contains blood, it follows that serum will remain in the supernatant fluid after centrifugation. This fluid could not be used for protein determinations, and if the amount of blood is significant, it probably could not be used for other tests. Cloudy spinal fluid is always centrifuged before chemical analysis, but for a different reason. Most constituents of normal spinal fluid are represented in lower concentrations than they are in blood (an exception is chloride, which is present in greater concentrations in spinal fluid than in blood). Hence, for most tests, serum will elevate the constituent being measured. The amount of blood present and the nature of the test could be used to decide whether or not a particular determination is valid. Less than ideal CSF specimens (icteric, hemolyzed, etc.) should not be indiscriminately discarded without first consulting the physician regarding the urgency of the case. Although the clinical laboratory does not control actual drawing of the specimen, you should do everything possible to aid the physician. For example, you might insure that specially cleaned screwcap tubes are used in the spinal puncture kit.

14-13. It will be rare indeed that you will be in a position to keep spinal fluid for any length of time. Tests on spinal fluid are usually ordered on an emergency basis, or at least with the intent of receiving the results within a matter of hours. Refrigeration is generally adequate limited preservation, except in the case of spinal fluid glucose. If bacteria are present, a spinal fluid glucose will drop markedly in a few hours. As stated before, cloudy spinal fluid should always be centrifuged. But this will not eliminate bacterial contamination, and the specimen cannot be saved as it could have been if it had been sterile.

14-14. You will need to know how much CSF is required for each procedure, because the quantity required is often less than that for blood or serum. Some technicians may assume that it is just a matter of substituting CSF for serum with no change in calculations. This is not so because the constituents of spinal fluid are present in amounts different from their occurrence in serum. A change in dilution necessitates a corresponding change in calculations.

15. Protein-Free Filtrates

15-1. Perhaps you have wondered why there are different types of protein-free filtrates (PFF). You may have even questioned the necessity of a PFF for various tests. The rationale, principle, and techniques involving PFF are discussed in this section.

15-2. **Rationale for PFF.** The primary purpose of preparing a PFF is to remove most of the protein from a body fluid so that protein will not interfere with the tests to be performed. Preparation of a PFF also eliminates other interfering substances such as pigments. For example, it is known that a high serum bilirubin intensifies color in the Lieberman-Burchard reaction for serum cholesterol. A simple and convenient way to eliminate this problem is to prepare a PFF. In other situations, the value of a PFF may be less apparent to you. In the Schales chloride determination, a PFF is recommended primarily because mercuric nitrate can react with protein. If mercuric ions react with protein, quantitative aspects of a chloride titration can be adversely affected. In spite of this fact, many laboratories perform the chloride on serum, with reportedly good results. As you can see, in some tests it is absolutely essential to prepare a PFF. In other determinations, preparing a PFF has certain technical advantages. Preparing a PFF takes a little time. With the current use of rapid and stable precipitating reagents, you would usually prepare a filtrate whenever one is recommended. For a list of the common filtrates and their characteristics, see table 7.

15-3. **Principles of Protein Precipitation.** It is because of the chemical nature of proteins that we shall explain why they are precipitated from solution. Proteins are composed of large molecules and behave as colloids in solution, or more correctly stated, in suspension. Proteins are made up of amino acids. At one end of a protein

TABLE 7

DATA ON PROTEIN-FREE FILTRATES

Method	Reagents	Remarks
Folin-Wu	10 percent tungstate + 2/3 N sulfuric acid	Not for Somogyi true glucose or phosphorus.
Somogyi	10 percent zinc sulfate + 0. 5 N sodium hydroxide	Not for NPN, phosphorus, or uric acid.
TCA	10 percent trichloroacetic acid	Preferred for phosphorus and O-Toluidine glucose.
Haden	$\frac{N}{12}$ sulfuric acid + 10 percent sodium tungstate	Not for Somogyi true glucose or phosphorus.
STA	Water + 10 percent sodium tungstate + 2/3 N sulfuric acid + phosphoric acid as a stabilizer. Mixed in order.	Not for Somogyi true glucose or phosphorus.

molecule is a free amino group, and at the other end is a free carboxyl group. Other groups can occur at various places along the chain. This results in a net charge density on the protein molecule. Whether protein behaves as an acid or a base depends upon the pH of the solution in which the protein occurs. The solubility of a protein depends upon the attraction between protein molecules and molecules of the solvent. The point at which proteins precipitate from solution is determined by charge characteristics on the protein molecule; it follows, therefore, that a change of pH of the solution would affect protein solubility. This basic knowledge enables us to discuss certain types of precipitating substances.

15-4. *Type I precipitation.* Miscible solvents may be used. The addition of solvents which are less polar than water facilitates precipitation of protein. They dehydrate protein by competing for the water molecules. Alcohols, ether, and acetone are typical examples of this group of precipitating agents. They are recommended for the extraction of cholesterol with simultaneous precipitation of protein.

15-5. *Type II precipitation.* Insoluble salt formation is a second type of protein precipitation. Acid precipitants form insoluble protein salts, but whether or not heavy metals combine with the carboxyl groups of the protein is less certain. In any case, acids and metallic ions in certain concentrations do precipitate proteins dependent upon pH. In table 7 you can see three tungstic acid filtrates listed—Folin-Wu, Haden, and STA. The classic Folin-Wu and Haden filtrates are less often used today. The third filtrate is referred to as stable tungstic acid (STA). It has the advantage of stability for up to three years and as a one reagent solution. The Carraway modified STA uses phosphoric acid as a stabilizer. Two different strengths of STA reagent are used, one for whole blood and a weaker reagent for plasma or serum. A greater volume of very clear filtrate is obtained with trichloroacetic acid (TCA). This filtrate is advantageous when only a small quantity of specimen is available. As indicated in table 7, TCA filtrates are preferred for phosphorus determinations and glucose by the o-toluidine method. TCA filtrates have a lower pH than other filtrates listed in the table and will not precipitate the phosphorus with the protein. Among cationic precipitants, mercury and zinc are commonly used in the form of their salts. In this category of filtrates, several other metallic ions are also used. Cationic precipitants are more successful in removing saccharoids than are precipitants such as tungstic acids. For this reason, a zinc filtrate (Somogyi) is usually used instead of a Folin-Wu filtrate for true glucose. On the other hand, zinc may interfere with copper reoxidation. With some micro methods, the interference is significant, and zinc is not used as the precipitant. It must be kept in mind that certain components besides protein may precipitate with various types of precipitants. For example, zinc will precipitate more nonprotein nitrogen than will be precipitated by acid reagents.

15-6. *Performing the filtration.* In general, it is simpler to centrifuge a specimen than to filter it. However, there are many situations in which you might prefer to use filter paper. Normally, if a filtrate appears clear, it is suitable for most tests. It is true that to completely rule out turbidity it is not sufficient to merely look at the filtrate; however, most technicians accept the visual test of clarity. If filter paper is used, it is quite important that you have a knowledge of filter papers.

15-7. Filter papers vary widely in diameter, thickness, and porosity, and may be qualitative or quantitative. Qualitative filter paper is not usually used for PFFs. Quantitative filter paper is acid-washed by the manufacturer to remove as much mineral matter as possible, so that when the technician uses it no mineral matter from the filter paper will contaminate the PFF. Moreover, when the filter paper and precipitate are ignited and burned, the weight of ash from the filter paper should be so small as to be negligible for the usual gravimetric determination. Quantitative filter paper is made in degrees of fineness. There appears to be no conventional method of numbering filter paper relative to porosity. Generally, a large number designates a finer filter paper and a smaller number indicates a coarser filter paper. In regard to retentiveness, your choice of a particular grade of paper is governed by fineness of the precipitate.

15-8. The necessity for careful selection can be easily recognized when a filtrate becomes cloudy from the passage of very fine particles through filter paper pores which are too large. Precipitated proteins consisting of large, slimy agglomerates should be filtered through paper with relatively large pores. This affords filtration without letting any precipitate pass into the filtrate, and is especially useful with a Folin-Wu filtrate. A precipitate of medium size, such as calcium oxalate precipitated from a weak acid solution, may be filtered through paper of moderately fine texture, whereas barium sulfate, which usually tends to give very fine crystals, requires a dense paper with small pores. The denser or finer the filter paper, the slower the filtration will be; therefore, a very fine filter paper is used only when there is an actual need for it. A good point in technique to remember when filtering is to always pour the first few milliliters of filtrate back into the funnel, as a filtrate becomes clearer after a mat is formed.

15-9. Filter papers must be carefully protected from dust and laboratory fumes. They must occasionally be checked for contamination, especially contamination from chlorides and ammonia, by running a blank determination. Blank determinations are performed by substituting distilled water for the filtrate. Ammonia interferes with blood urea nitrogen determinations as well as with certain other tests. It is possible that in the manufacture of acid-washed filter papers, some ammonia salts may remain as an impurity. For the above reasons, filter papers used in the preparation of blood filtrates for nitrogen and urea determinations in which ammonia salts might interfere should be washed. You can do this by filtering 2/3 normal sulfuric acid several times, discarding the acid each time, and then washing the paper with nitrogen-free distilled water.

CHAPTER 6

Quality Control

STANDARDS AND PRECALIBRATED curves provide a means of calculating the value of an unknown specimen. However, calculation of a result does not insure the quality of that value. There may be a variety of factors which tend to invalidate the test result. There are always problems of reagent instability, contaminated glassware, instrument variation, and technician error. Even the most careful and experienced technician is not infallible and is subject to certain physical limitations (i.e., subjective comparisons of color, meniscus or galvanometer readings, pipetting, and others). Unless the doctor knows the precision of a result, he is not justified in accepting it as a basis for diagnosis or treatment. Yet, it is not possible for a doctor to analyze every item of laboratory data for accuracy, and indeed that is not his responsibility. Rather, you, as the laboratory technician must control the quality of your work within certain established limits.

2. Fortunately U.S. Air Force laboratories have always been under better control than many of their civilian counterparts because they use area surveys, adequate supervision, and rigid controls. In testimony before a U.S. Senate investigating committee considering recommendations for legislated controls, it was reported that "serious deficiencies have been demonstrated to exist in the nation's clinical laboratories."[3] Based on a study by The National Communicable Disease Center, it was pointed out to the committee that "erroneous results are obtained in more than 25 percent of all tests analyzed by these studies."[4] In a recent study of 328 neonatal deaths, one or more laboratory factors were identified in 34.5 percent of the deaths.[5] This is incriminating evidence indeed, and no laboratory is beyond the problems which exist.

3. Quality control is an attempt to insure reliability of the tests performed. What possible good is a test if the result is inaccurate? Unreliable results will mislead the doctor and quite possibly harm his patient. Both you and the doctor know there is always a degree of uncertainty with each result obtained. For example, if a cholesterol result is calculated to be 200 mg-%, it should be understood that it is not exactly 200, but may vary to some degree. If another, or even the same, technician repeats a test, a slightly different result will likely be obtained. This is where quality control comes in. It tells us the degree of uncertainty which always goes with a result. The object of a good quality control program is to keep this degree of uncertainty within narrow limits. As is the case in industry and in other sciences, the clinical laboratory has adapted certain statistical procedures which control the accuracy of results. All data submitted by the laboratory should be substantiated by sufficient statistics to prove that it is within reliable limits. It must be accepted that the worth of a laboratory rests on its proficiency and the quality of its work. There is no substitute for statistical proof of a result.

16. Essentials for Quality Control

16-1. You should have been impressed in this text and in your daily work with the need for a quality control program. Yet you may not have realized that quality control is a science in itself. As is true in any science, terms used must have exact meanings and the language is principally one of mathematics. In this section you will have an opportunity to review the necessary elements of a quality control program.

16-2. **Standards and Controls.** There is often confusion in the use of the terms "standard" and "control." A standard is a sample of exact known composition which is used to calculate the result of a sample of unknown composition. A prepared curve is a graphic representation of a standard or series of standard results. The value of a standard must obviously meet two basic requirements. First, it must be exact. A range is not suitable if the calculation is to result in an exact figure. For example, a common ruler may be considered a standard. If it is used to measure the top of a desk, the result is expressed in terms of the known length of the ruler, in units such as inches. Suppose you measure the desk at 3 ruler lengths, or 36 inches. Suppose the ruler is not exactly 12 inches long, but approximately 10 to 14 inches. The calculated result would also be approximate; the desk you measured could actually be 30 to 42 inches long. You can readily see why a standard must be exact. Second, the standard value must be known. There should never be a question in your mind as to the value of a standard. If you think that your ruler is 12 inches long but do not know this for certain, the measurement is also uncertain. Your answer should be expressed in a number of significant figures commensurate with calculations and procedures involved in preparation of the standard and in the procedure itself. To expand the example of a ruler, you would not expect to measure an item to the nearest tenth of an inch with a ruler that was not graduated in tenths of an inch. It is not always necessary to run a chemical standard through all steps of a test. For instance, the final color produced may be compared with a color chart or colored solution which would be considered the standard. A standard is frequently a pure chemical in a suitable solvent and does not usually contain extraneous material.

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[3] Based on testimony to the Senate subcommittee regarding Medical Restraint of Trade Bill (S. 260).

[4] Testimony by Dr. David J. Spencer, Director of the National Communicable Disease Center to the Senate Committee *(ibid.)*, as reported in *Medical News*, Vol. 1, No. 9, Feb. 27, 1967.

[5] Dr. Spencer, *ibid.*

16-3. You have worked with many chemicals in the clinical laboratory. No doubt you realize that there are degrees of chemical purity, each with its own variations. Perhaps we should clarify what is understood by the term "chemical purity." We refer to A.C.S. grade chemicals, U.S.P., N.F., technical, and reagent grades. In addition, there are specific purity requirements for particular chemicals used in specific tests. These terms are often confused, so let us first define them.

a. A.C.S. (American Chemical Society) grade is the only universally accepted standard of chemical purity. This A.C.S. designation is assigned if a chemical assay passes American Chemical Society specifications for purity.

b. U.S.P. (United States Pharmacopeia) is a grade designation assigned by the U.S. Pharmacopeia Convention primarily on the basis of therapeutic value. U.S.P. chemicals usually are not of sufficient chemical purity for use as reagents in clinical chemistry.

c. N. F. (National Formulary) is also a grade designation assigned on the basis of therapeutic value, and, thus, is useful in pharmacology. N.F. grade chemicals are not pure enough for general use in clinical chemistry reagents either. This designation is assigned by the Committee on National Formulary by authority of the American Pharmaceutical Association.

d. Technical grade chemicals are manufactured primarily for industrial use. This grade of chemicals is of reasonable purity for commercial processes but unsuitable for use in quantitative chemistry because of contaminants.

e. Reagent grade chemicals are chemicals manufactured for use as reagents. This designation is assigned to various chemicals by individual chemical companies and the designation may or may not meet specific reagent requirements. In some cases, a company may certify a certain chemical or reagent for use in a specific method of analysis. This certification is usually valid when it is made by a reliable manufacturer. It always relates to a particular test and does not imply chemical purity other than the minimum allowable in the specific test procedure. Generally, published methods state the chemical purity limitations when they are a factor in the reaction. If the requirements are not stated, it is assumed that the most pure chemical available will be used.

16-4. The two basic types of standards are (1) primary standards and (2) secondary standards. Primary standards consist of the purest form of a chemical substance dissolved in a suitable solvent. The lack of contaminants in a primary standard is not completely desirable, however, because a pure standard does not react entirely as a test specimen which contains inhibitors or other contaminants. Some primary standards are available commercially which contain extraneous substances, but meet the basic definition of a standard in containing a pure, weighed quantity of the chemical to be assayed. Secondary standards are not chemically identical to the substance assayed, but they are related through some physical or chemical property which makes them useful as standards. An ordinary color comparison chart could be considered a secondary standard. It should be reiterated that use of a standard in no way verifies or controls a result. An accurate standard may be used in a manner which results in completely unreliable results. For example, if a miscalibrated spectrophotometer is used, the quality of the standard will be invalidated even though the standard is most carefully prepared. The same is true of a prepared curve, with the added danger that a curve which is not checked each time the procedure is performed may not hold under conditions which have changed since the curve was established.

16-5. A control is a specimen of known approximate value which is treated in the same way as the unknown specimen. The range or value for a control should ideally be close to that of the specimen being assayed. For example, a group of unknown bilirubin samples with values near 15 mg-% should be run with a control near 15 mg-%. One reason is that a control of lower value would not prove that a high result could be achieved. A control obviously differs from a standard in various ways. Ordinarily, control serum has properties similar to the properties of the unknown specimen. It may contain protein and other constituents which are present with the unknown. If a procedure calls for preparation of a protein-free filtrate and the precipitating reagent is not working properly, it would accomplish very little to use a control which is free of protein. Further, a control is not necessarily assigned a definite value, and the constituent being controlled may not have been accurately weighed. Where the constituents of a control specimen have been accurately weighed and dissolved in a suitable solvent, the concentrations given are referred to as "weighed-in" values. However, if the concentrations of constituents are determined from pooled samples of unknown concentration, the values given for the control serum are called "assayed" values. Both values depend upon the percent recovery of the specific assay method used. Therefore some notice should be taken of the method used to quantitate constituents of the standard. Ideally, the assay method should be the same as the method you wish to control. This is not always possible, but information can be obtained by comparing the confidence limits of different methods. There should be no doubt that standards and controls have different purposes and cannot be interchanged. If a calculation is based on the value of a control, the control does not automatically become a suitable standard. A solution which does not contain an exact known amount of the constituent in a suitable solvent falls short of the definition reserved for a standard.

16-6. **Calculating Results.** Prepared curves may give you a false sense of security. Analogous to the use of a prepared curve is the use of a factor which is used knowingly or unknowingly in a calculation. The use of a factor is not justified unless you understand its derivation. A laboratory which, as a matter of policy, takes a particular reading and multiplies or divides it by a factor should carefully evaluate its procedures. It has always been considered sound scientific practice to document every calculation in such a way that it can be referred to at any time. The use of a neat data sheet is the accepted method of doing this. Scraps of paper which are copied over and discarded are not acceptable. A technician should be in a position to recalculate his results or examine any part of the mathematics involved. Instrument readings should be recorded as they are taken. Erasures should not be made, because they may make the worksheet illegible. It is also possible that a supposed error is not really an error. An incorrect result should be neatly lined through and the correct figure should be entered. Copying a data sheet merely invites additional errors and results in the loss of valuable data. All tests should be performed simultaneously with standards and controls carefully documenting each recordable result. In some cases, a single standard is not adequate because color development may not follow Beer's Law at various concentrations.

16-7. **Instrumentation.** One major advantage of a quality control program is the clue it gives to inadequate instrumentation. Spectrophotometers, in particular, should be checked carefully for calibration errors. Variations in cuvettes and electrical fluctuations must be recognized. Proper use of controls will make inadequacies apparent, but daily instrument checks are required. With the Coleman, Jr. spectrophotometer used in most U.S. Air Force facilities, the check should include exciter lamp, wavelength calibration, lenses, zero setting, and 100 percent setting. We gave a detailed explanation of the spectrophotometer check in an earlier chapter. A didymium filter is available for wavelength calibration. If calibration of an instrument changes, it is necessary to check all precalibrated curves which are used with the particular instrument. Finally, limitations of an instrument must be known. Results cannot be achieved with an instrument that was not designed with the degree of accuracy sought. For example, as explained in Volume 2, the pH of blood would not be measured with just any pH meter. In fact, only relatively expensive, specially designed instruments can be used for that purpose. To use any other meter would yield results that have no clinical value.

16-8. **Accuracy, Precision, and Validity.** If you achieve a value which is within the allowable variation established for a particular determination, it is considered *accurate*. It is another matter to consistently achieve accurate results. The concept of duplicating a result within the allowable range in two or more determinations is termed "precision" (reproducibility). It is emphasized that accuracy and precision are not synonymous. It is possible to achieve accuracy without precision, and it is possible to have precision without accuracy. For example, assume that the actual glucose value of a serum sample is known to be 100 mg-%. Duplicate determinations yield 80 mg-% and 120 mg-%, respectively. The average of 100 mg-% is indeed accurate, but results are not reproducible. The extent to which a result may vary from the established value is determined statistically, as explained in the following section. In any case, a variation of 40 mg-% (120 down to 80) for a glucose determination does not appear to be within acceptable limits. A second possibility is precision without accuracy. Obviously, a laboratory could perform a particular procedure several times and consistently reproduce erroneous results. In the case of the glucose example, a result of 80 mg-% might be achieved on two or more consecutive determinations. This is not nearly as absurd as it may seem. A laboratory using reagents which are incorrectly prepared or deteriorated could repeatedly obtain inaccurate results. Poor individual technique, on the other hand, most often leads to a loss of precision.

16-9. Another term which may not be completely clear to you is "validity." A procedure is considered valid if it measures consistently what it is intended to measure. A procedure for alkaline phosphatase, for example, would not be valid if pH of the substrate is in a range of 4.8 to 6.0; however, it could well be reproducible.

16-10. A quality control program must be established with some knowledge of problems which can exist and with a working knowledge of exact terminology. The concepts of accuracy and precision encompass a variety of problems which must ultimately be identified. Be certain that you are able to cite an authority or current reference for every step of your procedure. Recognize, too, that references are sometimes in error and must be cross-checked and updated. Some factors responsible for poor quality may be listed as follows:

a. Faulty or inaccurately calibrated equipment, including glassware.
b. Poor technique.
c. Variation in test conditions, such as time and temperature.
d. Dirty glassware.
e. Incorrect curve, standard, or factor.
f. Poor method or method modification without standardizing for accuracy and reproducibility.
g. Unsuitable specimen.
h. Unsatisfactory reagents.
i. Contamination and interference.
j. Errors in observation and calculations.

16-11. **Mathematics.** A substantial number of errors which occur in clinical chemistry are traceable to errors in mathematics. We have already discussed the necessity of preparing and maintaining records of each procedure. The degree of automation will largely determine the nature of such records. In some cases, there may be little more involved than recording a result. If various mathematical procedures are involved there is always the possibility of error, even in simple arithmetic. As we stated earlier, science is most scientific when it is expressed mathematically. The technician who excuses his lack of facility in mathematics does not change the fact that clinical chemistry is expressed numerically. The ability to state a problem and express a result in mathematical language is invaluable. If practice is necessary, it should be understood that each of us must practice until he achieves proficiency.

16-12. One area which relates directly to mathematics and accuracy in reporting results is the concept of significant figures. Any figure which represents an actual measured quantity is a significant figure. A general rule is to identify the first and final figure of an expression which represents actual measured quantities. The number of digits between is equal to the number of significant figures without regard to decimal point. For example, a result which is reported as 1 mg. per ml. or 100 mg-% is expressed in one significant figure. If it is written as 100.5 mg-%, there are four significant figures. The key to identifying a figure as significant is to determine whether it represents a measured value. Zero is not significant if it appears as the first figure of a number, as in 0.1; but in the expression 1.0 there are two significant figures. You are encouraged to refer to a textbook of mathematics if the concept of significant figures is not clear. You will find it pays to be conscientious in submitting numerical reports in the correct number of significant figures. A glucose result which is reported to the fourth decimal is likely to cast a reflection on the laboratory which produced it. Further, since it is meaningless to report a result with a degree of accuracy that does not exist in the procedure, this should not be attempted.

17. Quality Control Program

17-1. If you have a genuine desire to maintain the highest quality in your work, it is not difficult to establish a quality control program. While we have limited our discussion of this type of program to its application in clinical chemistry, a quality control program may be established for any area of the clinical laboratory. We shall explain in this section how you can establish a program.

17-2. **Control Specimen.** It is important that enough control specimens be obtained or prepared to perpetuate a quality control program for several months during the initial evaluation phase. The volume required depends upon the sample volume necessary for each procedure you wish to control and upon the frequency of the test run. Creatinine, for instance, may be determined from a 2-ml. or 0.5-ml. sample depending upon whether a macro-technique or semimicro-technique is used. Some procedures may also be run infrequently. Regardless of frequency, a good quality control program should include a control in each run of specimens. Reliable commercial control serums are readily available in various unit volumes, assayed, unassayed, large or small lots, in normal or abnormal ranges, and for enzymes or special tests. The cost is nominal when it is considered that chemistry results are only as good as the quality control program which supports them.

17-3. A reliable serum pool may be used instead of commercial products. However, there are vital factors that must be considered before you attempt to prepare your own pooled serum. A stable, reliable, control pool cannot be maintained without specially trained technicians who have sufficient time to monitor and process the serum pool. A minimum of 2 L. should be collected before initial processing. Serum containing interfering substances (BSP dye, gross bacterial contamination, among others) should be excluded. Separate pools of serum must be maintained for enzymes, protein-bound iodine, and whenever above- or below-normal levels are desired. A freezer is required for holding and storing sufficient volumes of serum at −10°C. A household type refrigerator does not maintain this temperature. A blood bank centrifuge is usually required to adequately separate fibrin clots and other insoluble material from the serum pool. After the control pool has been collected, thoroughly mixed, and filtered, the control constituents must be critically assayed and adjusted to suitable concentrations before working aliquots are dispensed. It is obviously no simple matter to prepare your own control serum. The advantages and disadvantages should be weighed against your unique capability in personnel, equipment, time, and financial resources. The necessity of using quality control serum is undisputed, but the choice between commercial or locally prepared material will depend upon the factors mentioned.

17-4. **Statistical Expression.** Quality control implies a system for measuring the degree of precision in procedures. In its statistical context, quality control does not measure the accuracy of a procedure. It does measure variables including reagent and method reliability, technician skill, and instrumentation. Variables are estimated statistically in an expression of total allowable variation, termed "confidence limits." Confidence limits are established by calculating the dispersion of values for a test on either side of the average value. This mathematical measurement is termed "standard deviation." It is desirable that you have some knowledge of statistical frequency distribution. If a laboratory test is performed on a sufficiently large number of normal people and a distribution curve is plotted of the frequency of results, we would expect to obtain a typical bell-shaped curve. The peak of this curve would be at or near the average of all values obtained. The height of the curve would decrease as we moved away from the average in each direction. A sample curve is shown in figure 19. Standard deviation (SD) is calculated from the values which made the curve, by the following formula:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n - 1}}$$

where

σ is the standard deviation,
Σd^2 is the sum of the squared differences from the average, and
n is the number of test results used.

17-5. Standard deviation tells us that 68 percent of the values are in an area from the average −1 SD to the average +1 SD ($-\sigma$ to $+\sigma$). Likewise, 95.5 percent of the values are between -2σ and $+2\sigma$, and 99.7 percent from -3σ to $+3\sigma$. Usually two standard deviations, which represent 95 percent confidence limits, are used for laboratory work. Many laboratories use ±2.5 SD, which includes 98.8 percent of the values. This would allow for a possible 12 outlying values for every 1,000 determinations. How can we apply this to assure accuracy in the laboratory? This will require some effort and at least 3 weeks before practical results can be realized. The first step is to establish a standard deviation for each test which you want to control. After this, daily analysis of the control specimen described previously will instantly tell you if your results are within the confidence limits desired.

17-6. **Daily Records.** The calculation of standard deviation is based on a record of daily results which cover a period of not less than 15 days, and in most cases at least 21 days. A chart, as shown in figure 20, is very helpful. For purposes of illustration, the values for a series of potassium determinations have been entered in

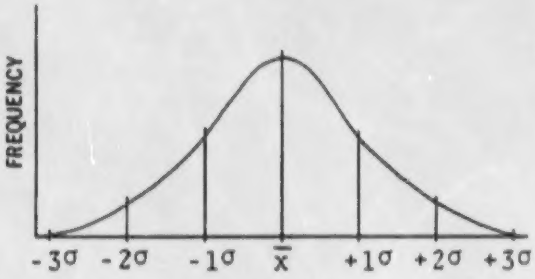

Figure 19. Frequency distribution curve.

figure 20. The sum of these values, divided by the number of tests, gives the average value. Note that the difference of each daily result from the average is entered in column 2. The difference is then squared, as shown in column 3 of this figure. The sum of the differences is entered in your data table and is applied in the standard deviation formula as shown. To complete the formula the number of daily results used, −1, is determined and is inserted in the formula as "*n*−1." The division quotient is calculated and finally the square root of the quotient is obtained. (A table of square roots is very useful here.)

17-7. Once the standard deviation is established, you can begin to control the quality of your work. Assume that the mg-% values for daily glucose procedures are charted and the standard deviation is calculated as 3 mg-%. These values may be placed on a graph, as indicated in figure 21. As each daily result (numbered consecutively) is entered on the graph, you will immediately know whether you are within the established confidence limits. For the range of values plotted in figure 21, one standard deviation unit represents 3 mg-%. It is important to realize that translation of SD units to mg-% depends upon the range of values used to establish the curve. For example, in figure 21 ±2 SD units would represent an allowable range of ±6 mg-% and that would be the allowable range for the control serum to be in control at a concentration of 100 mg-%. At a level of 300 mg-% the range of allowable error would be ±18 mg-%, not ±6 mg-%, because ±6 percent of 300 mg-% is ±18 mg-%.

17-8. Certain steps are necessary in analyzing the data you have recorded. If you are outside confidence limits, record your results and repeat the entire set of analyses, including another aliquote of the control. If the repeat results are in control, they may be reported; if not, the procedure, reagents, instrument, and your own performance should be checked for error. It must be emphasized that repeating out-of-control runs and investigation of the procedure in consistently out-of-control runs are essential operations. Data is best analyzed as it becomes available. Specifications which are becoming most widely accepted for analysis of quality control data impose the following requirements:

a. Determinations must be consecutive and must be performed on different days.

Daily Test Results	Differences From Average (d)	Squared Diff. From Average (d^2)
1. 5.7	0.1	0.01
2. 6.0	0.2	0.04
3. 5.9	0.1	0.01
4. 5.6	0.2	0.04
5. 5.8	0.0	0.00
6. 6.0	0.2	0.04
7. 5.7	0.1	0.01
8. 5.7	0.1	0.01
9. 5.9	0.1	0.01
10. 5.7	0.1	0.01
11. 5.7	0.1	0.01
12. 5.8	0.0	0.00
13. 5.7	0.1	0.01
14. 5.7	0.1	0.01
15. 5.9	0.1	0.01
Mean Ave ($\bar{x}$)		Sum (Σd^2)
= 5.8		= 0.22

Calculation of Standard Deviation

$$\sigma = \sqrt{\frac{\Sigma d^2}{\underline{n}-1}}$$

$$\sigma = \sqrt{\frac{0.22}{14}}$$

$$\sigma = \sqrt{.0157}$$

$$\sigma = 0.13$$

Figure 20. Data chart for entering test results and expressing standard deviation (values for potassium are entered).

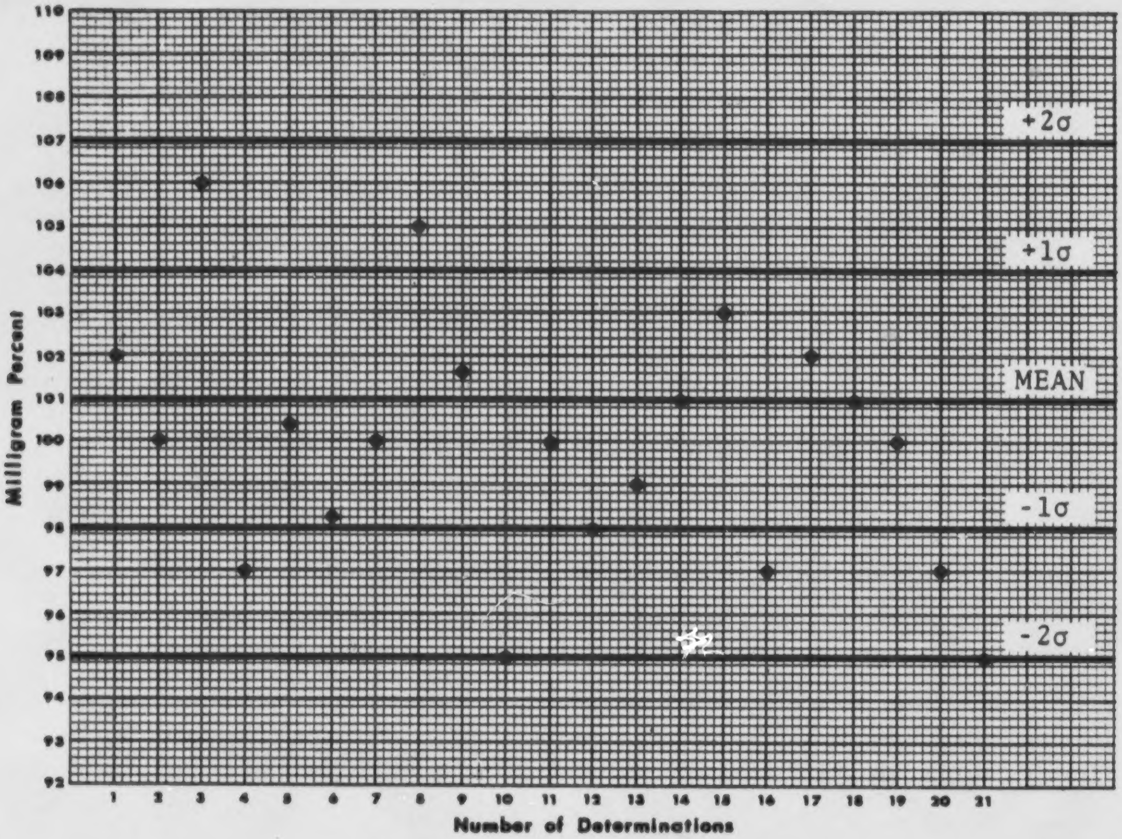

Figure 21. Quality control chart for glucose.

b. Each figure represents a single measurement, and no figures are discarded.

c. The pool of serum used in the program must meet certain standard requirements.

d. Most authorities do not accept less than 15 determinations for statistical purposes, and many require 21 as the minimum.

17-9. Problem Situation No. 1: You have determined a series of replicate analyses on a control serum for glucose. Calculate the average (mean) value for the series using the following values:

Specimen	mg-% glucose
1	118
2	115
3	111
4	114
5	117
6	117
7	120
8	116
9	112
10	117
11	122
12	119
13	117
14	117
15	121
16	121
17	121
18	117
19	111
20	115
21	119

17-10. Problem Situation No. 2: Calculate one standard deviation using the information in Problem Situation No. 1. The square root required may be found in table 8.

17-11. Problem Situation No. 3: What are the confidence limits for this glucose procedure, assuming an allowable error of 2 standard deviations?

17-12. Problem Situation No. 4: Plot the values of the tests given in Problem Situation No. 1 on graph paper furnished in the Handbook of Reference Material. Indicate the mean value and the confidence limits of the test by drawing horizontal lines at these levels of concentration.

17-13. Solution to Problem Situation No. 1: There were 21 replicate analyses for glucose. The sum of all values for glucose was 2,457. The average, or mean, value is obtained by dividing the sum of all values by the number (n) of determinations.

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TABLE 8

TABLE OF SQUARES AND SQUARE ROOTS

No.	Square	Square Root
110	1 21 00	10.48
111	1 23 21	10.53
112	1 25 44	10.58
113	1 27 69	10.63
114	1 29 96	10.67
115	1 32 25	10.72
116	1 34 56	10.77
117	1 36 89	10.81
118	1 39 24	10.86
119	1 41 61	10.90
120	1 44 00	10.95
121	1 46 41	11.00
122	1 48 84	11.04
123	1 51 29	11.09
124	1 53 76	11.13

$$x = \frac{\text{sum}}{n} = \frac{2457}{21} = 117$$

17-14. Solution to Problem Situation No. 2: You must calculate the differences from the average value (d) and the square of this difference (d^2) for each test value. For instance, the first value was 118 mg-%. The average value was 117 mg-%. The difference between 118 and 117 is 1, so this is the first number in the (d) column. The difference squared (d^2) column or (1^2) is also 1. The second specimen (115) differs from the average (117) by 2, so the second value in column (d) is 2 and in the (d^2) column the second value is (2^2) which is 4. Check your calculations with the answers below and correct any mistakes.

Specimen	*Test Results*	*(d)*	*(d²)*
1	118	1	1
2	115	2	4
3	111	6	36
4	114	3	9
5	117	0	
6	117	0	
7	120	3	9
8	116	1	1
9	112	5	25
10	117	0	
11	122	5	25
12	119	2	4
13	117	0	
14	117	0	
15	121	4	16
16	121	4	16
17	121	4	16
18	117	0	
19	111	6	36
20	115	2	4
21	119	2	4
	sum = 2457		d^2 = 206

$$\frac{\text{sum}}{n} = 117 = x$$

The formula for calculating standard deviation is:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

The number of tests (n) is known. You have just calculated the d^2 values. Add the d^2 column of figures to obtain the sum of d^2 or Σd^2. Substitute n and Σd^2 values in the standard deviation formula and solve for σ.

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} = \sqrt{\frac{206}{21-1}} = \sqrt{\frac{206}{20}} = \sqrt{10.3}$$

$$\sigma = 3.2 \text{ mg-\%}$$

17-15. Solution to Problem Situation No. 3: Confidence limits establish the total allowable variation of a method. Since 2 standard deviations are given as allowable variation, 2 × the standard deviation value (±3.2) = total allowable variations of ±6.4 mg-%. This allowable variation is valid only at the mean level of concentration (117 mg-% ±6.2 mg-% or from 111 mg-% to 123 mg-%).

17-16. Solution to Problem Situation No. 4: Figure 22 is the solution to this problem.

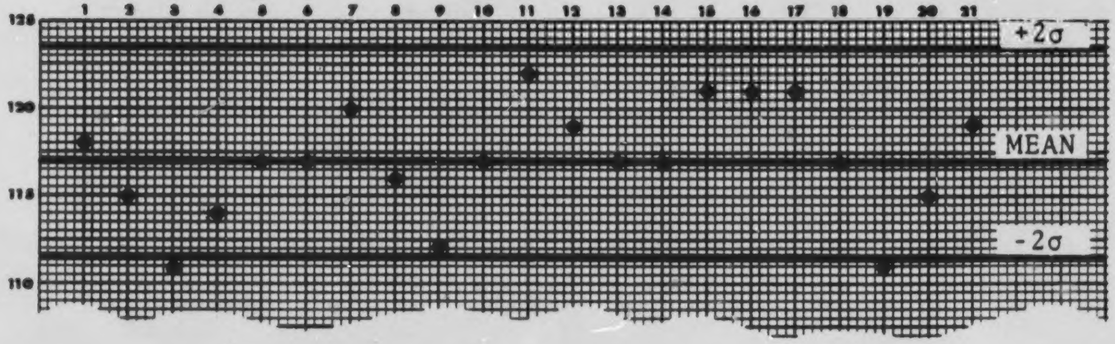

Figure 22. Solution to Problem Situation No. 4.

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17-17. A simple mathematical means of calculating allowable limits of error is represented in the following expression referred to as Tonks' formula. However, some chemists consider this formula overly generous in allowance. It should be pointed out in defense of the formula that, regardless of the calculated allowable error, Tonks did not permit an error greater than ±10 percent. This formula is not used for enzyme determinations. Tonks' formula is as follows:

$$\text{Percent error allowed} = \frac{\pm 1/4\ (\text{upper limit-lower limit})}{\text{mean value of the range}} \times 100,$$

or, reduced to simpler terms:

$$\text{Percent error allowed} = \frac{\pm 25\ (\text{upper limit-lower limit})}{\text{mean value of the range}}$$

Using a Folin-Wu glucose with a range of 80 to 120 mg-% as an example, the allowable percent error would be as follows:

$$\frac{\pm 25\ (120-80)}{100} = \frac{\pm 40}{4} = \pm 10 \text{ percent allowable error}$$

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ECI Form No. 17

STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 100 through 117

Guide Number	
100	Introduction to Medical Laboratory Career Field; The USAF Medical Laboratory Service; The USAF Medical Laboratory Career Field; pages 1-4
101	The Medical Laboratory Specialist/Technician; Medical Laboratory Safety Procedures; pages 4-6
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CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 2

Objective: To show the ability to define terms used in basic chemistry and to use this knowledge to write chemical formulas and interpret chemical reactions.

1. What basic particle, or particles, are contained in the atomic nucleus of the lightest isotope of hydrogen? (5-2)

2. What is the fundamental proposal in Dalton's atomic theory? (5-2)

3. Of what value is a knowledge of orbital electron population? (5-4)

4. What is the maximum number of electrons that could occupy the third energy level, or M shell? (5-4)

5. Where is the mass of an atom concentrated? (5-6)

6. How is it possible that atomic charts show the chemical atomic weight of an element as a decimal, though mass is an expression of protons and neutrons? (5-8)

7. What is an isotope? (5-8)

8. How do compounds differ from mixtures? (6-3)

9. When mixed, alcohol and water intermingle and produce heat. The water and alcohol can be separated by distillation. Is this a mixture or a compound that is formed? (6-3)

10. What is meant by *valence*? (6-6)

11. Why is radon called an *inert* gas? (6-7)

12. What is the oxidation number of silicon in the compound SiO_2? (6-8,9)

13. What is the oxidation number of free nitrogen? (6-9)

14. A sample of sodium trititanate yields .05 g. atoms of sodium, .075 g. atoms of titanium, and 0.175 g. atoms of oxygen. What is the formula? (6-12)

15. How does a coordinate covalent bond differ from a simple covalent bond? (6-14,15)

16. What is oxidation? (6-23)

17. What are tribasic acids? (6-32)

18. How can a substance which does not contain hydroxyl ions, such as ammonia, "furnish" hydroxyl ions? (6-33)

19. What is organic chemistry? (7-1)

20. Characterize or define the following classes of compounds.
    (1) Hydrocarbons.
    (2) Alcohols.
    (3) Amino acids.
    (4) Proteins.
    (5) Carbohydrates.
    (7-2,3,7,8)

CHAPTER 3

Objectives: To define terms used for solution concentration; to use principles involved in the preparation of quantitative solutions; and to perform related calculations.

1. Define the following terms:
    a. Solution.
    b. Solvent.
    c. Solute.
    d. Saturated solution.
    (8-2,3)

2. How many milliliters of acid are used to make 100 ml. of a 10 percent *V/V* solution? (9-2,3)

3. A procedure requires 69.8 g. of anhydrous copper sulfate ($CuSO_4$). All you have is hydrated copper sulfate ($CuSO_4 \cdot 5H_2O$). How many grams of the hydrated copper sulfate would you use? (9-4)

4. How many equivalent weights are there in a 1*M* solution of H_3PO_4? (9-4–6)

5. How many grams of sulfuric acid (H_2SO_4) are needed to prepare 200 ml. of a $1N$ solution? (9-6)

6. How many grams of HCl are necessary to prepare 200 ml. of $0.001N$ HCl solution? (9-6)

7. How many milliliters of concentrated HNO_3, specific gravity 1.4 and 70 percent pure, are necessary to prepare 500 ml. of $0.1M$ HNO_3? (9-6)

8. How many milliliters of concentrated sulfuric aicd, specific gravity 1.84 and 95 percent pure, are necessary to prepare 100 ml. of a $2M$ solution? (9-5,6)

9. How many milliliters of concentrated HCl having a specific gravity of 1.19 and 38 percent pure are necessary to prepare 500 ml. of $1.5N$ HCl? (9-6,7)

10. (a) How many milliliters of 85 percent alcohol are required to prepare 100 ml. of 65 percent alcohol?
    (b) How many milliliters of 3 percent sugar solution can be made from 600 ml. of 15 percent sugar solution?
    (9-12,13)

11. How many milliequivalents per liter are numerically qual to 311 mg-% of phosphorus? (9-14,15)

12. How should 116 volumes percent of carbon dioxide be reported in mEq/L? (9-15)

13. What is the hydroxyl ion concentration of an acid of pH 4? (10-2)

14. At what ratio of acid to salt is a buffer most efficient? (10-5)

15. If 40 ml. of an acid neutralize 80 ml. of $0.5N$ base, what is the normality of the acid? (10-8)

16. What is the normality of NaOH if 10 ml. of it neutralize 5 ml. of $1M$ H_2SO_4? (10-8)

17. If 5 ml. of 10*N* HNO_3 added to 10 ml. of water neutralize 25 ml. of base, what is the normality of the base? (10-8)

18. When titrating an unknown base against 0.1*N* HCl, it is found that three successive titrations require 6.30, 6.35, and 6.25 ml. of the base to neutralize three 5 ml. portions of the acid. What is the average normality of the base? (10-8)

19. Twenty milliliters of an acid are neutralized with 30 ml. of 0.5*N* base. What is the normality of the acid? (10-8)

20. If 5 ml. of 0.1*M* H_2SO_4 are neutralized with 15 ml. of base, what is the normality of the acid? (10-8)

CHAPTER 4

Objective: To define terms used in the physics of light as it relates to spectrophotometry and to perform calculations involved.

1. Assuming that the principle of a particular chemical test is valid, what determines how accurately you are able to relate color intensity to the component to be measured? (Introduction)

2. Cite one line of evidence that light is a series of expanding wave fronts. (11-2)

3. How is a prism able to separate component wavelengths of light? (11-2)

4. How is a wavelength measured? (11-2)

5. State the Beer-Lambert law. (11-3)

6. Give two situations in which colorimetry is used. (11-4)

7. What percent of the light energy which enters a blank solution will pass through and be indicated on the galvanometer scale? (11-5)

8. Why does a red solution appear red? (11-6)

9. For what is a diffraction grating used? (11-6)

10. Determine the concentration of the unknown.
    a. 2 ml. of a 20 mg-% std. used $OD_s = 0.200$
    $OD_s = 0.200$
    2 ml. of a 1: 10 STA filtrate used.
    $OD_u = 0.400$
    b. 0.5 ml. of a 10 mg-% std. used.
    $OD_s = 0.350$
    0.5 ml. of serum used.
    $OD_u = 0.700$
    c. 5 ml. of a 0.2 mg-% std. used.
    $OD_s = 0.325$
    5 ml. of a 1: 10 STA filtrate used.
    $OD_u = 0.525$
    d. 4 ml. of a 0.5 mg-% std. used.
    $OD_s = 0.800$
    4 ml. of a 1: 10 STA filtrate used.
    $OD_u = 0.400$
    (12-2,3)

11. If 1 ml. of serum is used in a test to make a 1: 10 PFF, and 2 ml. of the PFF are used, what is the *V* factor? (12-3)

12. What is the purpose of the *V* factor in the spectrophotometer formula? (12-3)

13. Of what value is a nonlinear C-A curve? (12-13,14)

14. Using the graph paper provided in the Handbook of Reference Material, prepare a spectral-transmittance curve from the following values.

Percent Transmittance	*Wavelength*
36.0 % T	400 mμ
51.0 % T	425 mμ
66.0 % T	450 mμ
58.0 % T	475 mμ
46.0 % T	500 mμ
36.0 % T	520 mμ
33.5 % T	525 mμ
32.0 % T	530 mμ
30.0 % T	540 mμ
30.0 % T	550 mμ
33.8 % T	560 mμ
40.0 % T	575 mμ
88.0 % T	675 mμ

(12-14,15)

15. At which wavelength is a colored solution usually read? (12-15)

16. What determines the category of a cuvette? (12-16)

17. (a) In spectrophotometry, how do you know when the exciter lamp check is satisfactory? (12-18–20)
    (b) What range of error is allowable in the lamp darkening check of a Coleman, Jr. spectrophotometer? (12-18–20)

18. There are certain prerequisites in spectrophotometry for obtaining an initial percent T reading for the lamp darkening check. What are the requirements for an exciter lamp? (12-20)

19. What component of the flame photometer aspirates specimens into a flame? (13-4)

CHAPTER 5

Objective: To demonstrate knowledge of the collection and preparation of blood and cerebrospinal fluid specimens for chemical analysis.

1. Airman Brown forgot to draw blood for a BUN determination, but drew only a serology on a patient. The error was discovered just as the blood had clotted, and the procedure called for whole blood. How would you solve this problem? (14-2)

2. The nurse on Ward A forgot to withhold a patient's breakfast on whom a physician had ordered electrolytes, BUN, and a PBI. The patient is to be discharged from the hospital at 1000 hours; it is now 0800 hours, and results are needed before he is discharged. What would you recommend? (14-4)

3. The night call technician forgot to freeze a serum specimen which he had drawn for a transaminase at 2400 hours. It is 0800 hours the next day, and you discover the specimen in the refrigerator with the serum separated, but unfrozen. What would be the best thing to do? (14-7)

4. Which of the three tubes submitted to the laboratory for spinal fluid analysis is used for chemistries? (14-11)

5. What is the first thing that should be done when the laboratory receives CSF tubes? (14-11)

6. Each of three tubes submitted for CSF analysis contains a "tinge" of blood. Would it be best to return the request slip marked "specimen unsuitable," report the results, or ask the physician's opinion? Explain your answer. (14-12)

7. For what purpose is a PFF prepared other than to eliminate protein? (15-2)

8. What is the reasoning behind objecting to protein in the Schales chloride test? (15-2)

9. What is the chemical nature of proteins? (15-3)

10. Why do you suppose that a TCA filtrate is used for phosphorus? (Table 7; 15-5)

11. Name the type of filtrate discussed in the text which is described by each of the following phrases.
    a. 10% Na_2WO_4 + 2/3*N* H_2SO_4.
    b. A stabilized reagent.
    c. Used for phosphorus.
    d. For true glucose.
    e. Modified Folin-Wu.
    (Table 7, 15-5)

12. Name a common type of filtrate which uses a metallic cation? (15-5)

13. How does No. 3 filter paper usually compare with No. 4 filter paper? (15-7)

14. What is the main reason for using acid-washed filter paper in the clinical laboratory? (15-9)

CHAPTER 6

Objective: To show knowledge of the basic information necessary for maintenance of quality control in clinical chemistry.

1. Why should a technician, who is experienced and careful, be expected to substantiate his test results statistically? (Introduction)

2. Specifically, what do quality control statistics tell the physician or technician? (Introduction)

3. Does a color chart generally meet the requirements of a standard, or would it be more desirable to have a standard which can be treated as the unknown? (16-4)

4. To what extent does a standard control the precision of a result? (16-4)

5. What is the principal objection to using a prepared curve as a standard? (16-4)

6. May a control be used as a standard? (16-4,5)

7. Why is a control described as being of known "approximate value"? (16-5)

8. Why are multiple standards used? (16-6)

9. If control values are achieved, is it necessary to check the calibration of the instrument, e.g., a spectrophotometer? (16-7)

10. List four checks which should be completed on a Coleman, Jr. spectrophotometer as part of a quality control program. (16-7)

11. Distinguish between accuracy and precision. (16-8)

12. Why is it desirable to report a potassium result in two significant figures? (16-12)

13. How many significant figures in each of the following?
    a. 6.0×10^3
    b. 6×10^4
    c. Product of 2.0×6.34
    d. Quotient of $\frac{2.4}{8.69}$
    (16-12)

14. What factors should you consider in order to determine whether to use commercial serum or your own control serum? (17-2,3)

15. What is meant by "confidence limits"? (17-4)

16. Assuming random distribution and a statistically adequate number of cases, what percent of the values will fall within 1 σ below the mean? (17-5,6,8)

17. In the analysis of quality control data, should figures which are obviously out of range be recorded or discarded? (17-8)

18. In 20 consecutive determinations, the sum of the squared difference is 159. What is the standard deviation? (17-14)

19. The normal range of a procedure is 10 to 18 mg-%. What is the allowable limit of error in percent, according to Tonks? (17-17)

CHAPTER 2

1. Of the three fundamental particles discussed, only one proton is found in the nucleus of the lightest isotope of hydrogen.

2. Dalton proposed that matter is composed of discrete units or particles which we call atoms.

3. A knowledge of orbital electron population enables the chemist to predict an element's chemical behavior as encompassed in the concept of valence.

4. $2(3)^2$ or 18.

5. The mass of an atom is concentrated in the nucleus.

6. In determining the atomic weight of an element, the atomic weights of the isotopes are averaged, and this may result in a decimal.

7. An isotope is a form of an element which differs from the other forms in atomic mass or in the number of neutrons.

8. Compounds represent elements which have been united chemically, whereas mixtures are united physically and can be physically separated.

9. A mixture.

10. Valence is the combining capacity of an element.

11. The valence ring of radon is complete and, hence, it does not readily give up, receive, or share electrons.

12. +4.

13. Zero.

14. $\text{Na} = \frac{.05}{.05} = 1;\ \text{Ti} = \frac{.075}{.05} - 1.5;\ 0 = \frac{.175}{.05} = 3.5$
Smallest multiple = 2, resulting in $Na_2Ti_3O_7$.

15. In coordinate covalence, one atom donates the electrons, while in the simple covalent bond each atom donates electrons to be shared.

16. Oxidation is the loss of electrons in a reaction.

17. Acids which furnish three hydrogen atoms per molecule are termed "tribasic."

18. Ammonia reacts with water to produce OH ions which are furnished by the water.

19. Organic chemistry is the study of carbon compounds.

20. (1) Hydrocarbons are compounds composed solely of carbon and hydrogen.
    (2) An alcohol is a hydrocarbon chain (R) and one or more OH groups.
    (3) Amino acids are organic compounds containing an amino group and an acid group.
    (4) Proteins are polymers of amino acids.
    (5) Carbohydrates are polyhydroxy aldehydes, ketones, or organic compounds which yield these substances on hydrolysis.

CHAPTER 3

1. *a.* A solution is a homogeneous mixture of two or more substances.
   b. A solvent is the medium in which a substance is dissolved.
   c. The solute is the substance which is dissolved.
   d. A saturated solution is a solution which contains all of a solute it can normally contain at a particular temperature and, in the case of gases, under a particular pressure.

2. 10 ml.

3. $\frac{69.8}{159.6} = \frac{x}{249.7}$

   $x = 109$ g.

4. 3, because total positive valence = 3.

5. $\frac{98}{2} \times \frac{200}{1000} = \frac{49}{5} = 9.8$ g.

6. The mol. wt. of HCl is 36.47

$$.001N = \frac{36.47}{1000}$$
$$\text{g/L} = .036 \text{ g/L}$$
$$\frac{200}{1000} = \frac{x}{.036} \text{ or } \frac{1}{5} \times .036 = .007 \text{ g.}$$

7. The mol. wt. of HNO_3 = 63.02.
500 ml. 0.1M = 1/2 × 0.1 × 63.02

We want: $\frac{3.15}{1.4}$ g. × $\frac{100}{70}$ = x ml. needed
$x = 3.2$ ml.

8. The mol. wt. of H_2SO_4 is 98.

100 ml. of 2M = $\frac{1}{10} \times 2 \times .98 = 19.6$

$\frac{19.6}{1.84}$ g. × $\frac{100}{95}$ = x ml. needed
$x = 11$ ml.

9. 500 ml. of 1.5N = $\frac{500}{1000}$ or (.5)
(0.5) (1.5 × 36.47) = 27.35 g.
$\frac{27.35}{1.19} \times \frac{100}{38}$ = 60.5 ml. needed
($\frac{2735}{45.22}$ = 60.5 ml.)

10. (a) $85x = 100 \times 65$
$x = 76.5$ ml.
(b) $(600)(15) = 3x$
$x = 3000$ ml.

11. $\frac{31}{1.8}$ = 17.2 = corrected eq. wt. in mg.
$\frac{311 \times 10}{17.2}$ = 181 mEq/L

12. $\frac{116}{2.2}$ = 53 mEq/L.

13. pH of 4 = 1×10^{-4} [H+]
$(1 \times 10^{-14}) - (1 \times 10^{-4}) = 1 \times 10^{-10}$ moles/L.

14. 1: 1.

15. $40x = (80)\ (.5)$
$x = 1N$

16. $1M\ H_2SO_4 = 2N$
$(5)\ (2) = 10x$
$x = 1N$

17. $(5)\ (10) = x(25)$
$x = 2N$ (The water is of no significance.)

18. The average of the titration is 6.3 ml.
$N_1 \times Vol_1 = N_2 \times Vol_2$
$(0.1)\ (5) = (6.3)\ (x)$
$x = \frac{.5}{6.3}$
$x = 0.08N$

19. $Vol_1 \times N_1 = Vol_2 \times N_2$
$(20)\ (x) = (30)\ (.5)$
$x = \frac{30 \times .5}{20}$
$x = 0.75N$

20. $0.1M = 0.2N$. The quantity of base used is not pertinent to the problem.

CHAPTER 4

1. The instrument you are using, e.g., a spectrophotometer, determines this.

2. One light energy interferes with another.

3. A prism is able to separate component wavelengths of light because the velocity of light through a solid varies with the wavelength. This results in unequal bending of the rays.

4. A wavelength may be measured between either maximum points or minimum points.

5. The Beer-Lambert law states that optical density of a colored solution is directly proportional to the depth of the solution through which the light passes as well as to the concentration of the color component.

6. It is used in specialized situations and in field laboratories.

7. Something less than 100 percent because some light energy at any wavelength is absorbed. However, for purposes of measurement, the scale is adjusted to 100 percent.

8. Because it *transmits* most of the light energy in the red area of the spectrum.

9. A diffraction grating is used as a monochromator.

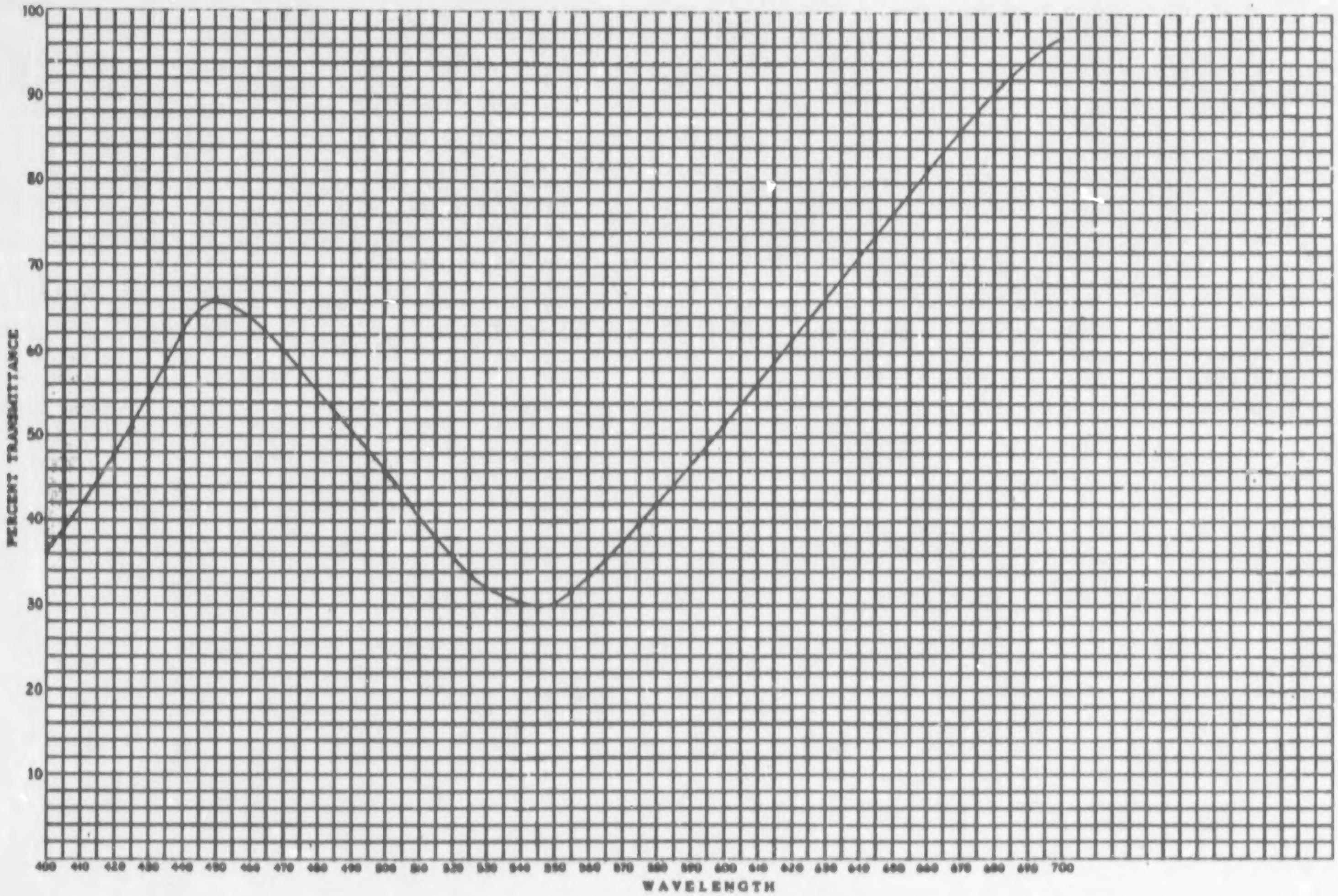
10. a. $\frac{.4}{.2} \times .4 \times \frac{100}{0.2} = 400$ mg-%
    b. $\frac{.700}{.350} \times .05 \times \frac{100}{0.5} = 20$ mg-%
    c. $\frac{.525}{.325} \times .01 \times \frac{100}{0.5} = 3.2$ mg-%
    d. $\frac{.400}{.800} \times .02 \times \frac{100}{0.4} = 2.5$ mg-%

11. 0.2.

12. The purpose of the *V* factor in the spectrophotometer formula is to relate the dilution of the unknown specimen to the amount of standard actually used in the test.

13. Depending upon the chemical principle of the test, a nonlinear C-A curve may be of value in calculating unknown results, just as a linear C-A curve may be used.

14. Your curve should appear as shown in CRE figure 1.



CRE Figure 1. Spectral Absorbance Curve

15. A solution is read at a wavelength which will show maximum absorption when the light passes through the solution.

16. Its optical properties.

17. (a) At 400 mμ the galvanometer needle will go past 100 percent T when the galvanometer controls are turned completely clockwise.
    (b) ± 1.5 percent T.

18. The lamp must be clean and new.

19. The atomizer.

CHAPTER 5

1. Use serum.

2. Perform the tests, and note under "Remarks" on the report that patient had breakfast, and comment whether serum was cloudy or clear.

3. Run the test; the specimen need not be frozen. It would not be advisable to draw another specimen since the physician would probably want to know what the transaminase had been at 2400 hours.

4. Tube number 2.

5. Be certain that the tubes are labeled.

6. Report the result, noting the amount of blood present under "Remarks" on the report. (It is generally unnecessary to place the burden for a technical decision on the physician, but it is acceptable and often desirable to discuss it with him.) According to the text, a "tinge" of blood would not significantly alter the results, if centrifuged, but it should not be completely dismissed either.

7. To eliminate other interfering matter, such as pigments.

8. Mercuric ions may bind with the protein.

9. Chains of amino acids.

10. TCA filtrate has a lower pH than other filtrates listed in table 7 and will not precipitate the phosphorous with the protein.

11. *a.* Folin-Wu.
    b. STA.
    c. TCA.
    d. Somogyi.
    e. Haden.

12. Somogyi filtrates use Zn+.

13. No. 4 filter paper has a finer texture than No. 3.

14. Acid washing removes ammonia salts which interfere with ammonia and nitrogen determinations.

CHAPTER 6

1. Because even the most experienced technician is not infallible. The doctor has a right to know the precision of results he is using for a patient. Only the laboratory technician is in a position to maintain this quality control.

2. Quality control statistics tell the degree of uncertainty associated with a result.

3. A color chart can be a kind of standard; it is not necessary to treat the standard as the unknown.

4. A standard does not attest to the precision of a result and does not function to control a procedure.

5. Prepared curves are not always checked each time a test is performed. They sometimes lead to inaccurate results because they are not adjusted to meet changes in the procedure or conditions of the test.

6. A control may be used as a standard if it contains an exact measured amount of the known substance.

7. "Approximate value" refers to the fact that the substance in question is not necessarily weighed into the control specimen.

8. Multiple standards rather than a single standard are particularly useful when a procedure does not follow Beer's law.

9. Yes; controls merely give a clue to inadequate instrumentation and should not be relied upon to check instrumentation because there may be compensating errors.

10. Exciter lamp, wavelength calibration, lenses, zero setting, and 100 percent setting.

11. Accuracy refers to the degree of variation of a result from the established value. Precision refers to the reproducibility of a result.

12. Because, as you know, the normal range for potassium in blood serum is approximately 4.0 to 6.0 mEq/L. This involves two significant figures.

13. *a.* 2.
    b. 1.
    c. 2.
    d. 2.

14. You should consider the cost of commercial serum and your unique capability in personnel, equipment, time, and financial resources.

15. "Confidence limits" implies range of total allowable variation.

16. 34 percent. Assuming random distribution and a statistically adequate number of tests, 1/2 of ±1σ units (68 percent of the values) is -1σ, or 34 percent of the values.

17. Recorded.

18. $\sqrt{\frac{159}{19}} = \sqrt{8.37} = 2.89$

19. $\frac{\pm 1/4\ (18 - 10)}{14} \times 100 = \frac{\pm 2}{14} \times 100\ \pm 14\%$. However, Tonks did not permit error greater than ±10 percent.

STOP–

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 1 PENCIL.

90411 01 22

VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed.* Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

3. (006) Ether, sulfuric acid, oxygen gas, and tuberculosis cultures in the laboratory indicate the diversity of the

a. safety hazards which can exist there.
b. duties in a laboratory career field.
c. systems used in a laboratory.
d. techniques carried on in a laboratory.

4. (007) Some atoms do *not* have

a. electrons.
b. protons.
c. neutrons.
d. nucleons.

5. (008) The maximum possible electron population in the L shell of an atom is

a. 2.
b. 4.
c. 6.
d. 8.

6. (008) Two isotopes of a particular element have the same

a. atomic mass.
b. atomic weight.
c. atomic number.
d. number of neutrons.

7. (010) A substance which is composed of two or more elements that are chemically combined in definite proportions *by weight* is called

a. an element.
b. a compound.
c. a mixture.
d. a solution.

8. (011) The number of electrons an element is able to gain, lose, or share is the element's

a. oxidation number.
b. atomic number.
c. ionization constant.
d. valence.

9. (012) Halogens usually have an oxidation number of −1 *except* when they are in combination with

a. hydroxide.
b. hydrogen.
c. oxygen.
d. nitrogen.

10. (012) What is the oxidation number of an element in the free state such as O_2?

a. −2.
b. −1.
c. 0.
d. +2.

11. (013) The analysis of a certain compound shows that it contains 0.05 *gram-atom* of sodium, 0.075 *gram-atom* of titanium, and 0.175 *gram-atom* of oxygen. The simplest formula for this compound is

a. $NaTi_2O_8$.
b. $Na_2Ti_3O_7$.
c. $Na_3Ti_4O_6$.
d. $Na_1Ti_5O_5$.

12. (014) Chemical bonding in which one atom donates all of unequally shared electrons is called

a. ionic bonding.
b. covalent bonding.
c. electrovalent bonding.
d. coordinate covalent bonding.

13. (015) The equation $Zn + CuSO_4 \rightarrow ZnSO_4$ is an example of

a. *combination* reaction.
b. *decomposition* reaction.
c. *single displacement* reaction.
d. *double displacement* reaction.

14. (015) What type of chemical reaction is represented by the equation $2H_2 + O_2 \rightarrow 2H_2O$?

a. The *combination* reaction.
b. The *decomposition* reaction.
c. The *single displacement* reaction.
d. The *double displacement* reaction.

15. (016) Which of the following states that *a system in equilibrium will react to a stress by establishing a new equilibrium?*

a. The law of conservation of mass-energy.
b. The law of multiple proportions.
c. The law of definite proportions.
d. LeChatelier's principle.

16. (107) Normally, the combination of any negative ion with any positive ion results in

a. a base.
b. a salt.
c. an acid.
d. a mixture.

17. (019) Which of the following does *not* have a cyclic structure?

a. A sterol.
b. Benzene.
c. A phenol.
d. Ethanol.

18. (019) Which of the following is an example of a disaccharide?

a. Starch.
b. Sucrose.
c. Fructose.
d. Dextrose.

19. (020) All solutions may be defined as

a. mixtures.
b. colloids.
c. liquids.
d. compounds.

20. (020) Which of the following is *not* a consideration in determining the solubility of a solid?

a. The nature of the solvent.
b. The pressure.
c. The temperature.
d. The nature of the solute.

21. (022) In double decomposition reactions, the equivalent weight of ferrous sulfate (F_eSO_4) is

a. half its molecular weight.
b. the same as its molecular weight.
c. twice its molecular weight.
d. not related to its molecular weight.

22. (023) How many milliliters of concentrated nitric acid (with a specific gravity of 1.4, an assay value of 70 percent, and a molecular weight of 63) are used to prepare 500 milliliters of a 1-N solution?

a. 1.6 milliliters.
b. 3.2 milliliters.
c. 16 milliliters.
d. 32 milliliters.

23. (023) How many grams of sulfuric acid (molecular weight, 98) are needed to prepare 300 milliliters of a 1-N solution?

a. 9.8 grams.
b. 14.7 grams.
c. 29.4 grams.
d. 39.2 grams.

24. (023) How many milliliters of a 10-percent solution of glucose are required to prepare 112 milliliters of a 5-percent solution?

a. 2.04 milliliters.
b. 5.6 milliliters.
c. 22.4 milliliters.
d. 56 milliliters.

25. (024) A value of 126 mg-% of sodium chloride is equivalent to

a. 20 mEq/L.
b. 40 mEq/L.
c. 60 mEq/L.
d. 80 mEq/L.

26. (025) Chemicals which resist a change in hydrogen ion concentration are referred to as

a. acids.
b. bases.
c. buffers.
d. indicators.

27. (026) A buffer is most efficient when the concentration of the acid is

a. one-third the concentration of the salt.
b. half the concentration of the salt.
c. equal to the concentration of the salt.
d. twice the concentration of the salt.

28. (030) Which of the following states that optical density of a colored solution is directly proportional to the concentration?

a. Newton's axiom.
b. Beer-Lambert law.
c. LeChatlier's principle.
d. Bronsted-Lowry concept.

29. (032) Instruments which measure radiant energy caused by photoluminescence are called

a. Duboscq colorimeters.
b. ultraviolet spectrophotometers.
c. fluorimeters.
d. infrared spectrophotometers.

30. (033) Which of the following should *not* invalidate Beer's law?

a. Clear solutions.
b. Heavy concentrations.
c. Tyndall effect.
d. Turbid solutions.

31. (033) Calculate the *V* factor for the spectrophotometer formula if you use 5 ml. of a protein-free filtrate (PFF) prepared as follows: 16 ml. acid, 2 ml. serum, 2 ml. tungstate.

a. 0.5.
b. 1.0.
c. 2.0.
d. 10.0.

32. (033) Given data as follows:
5 ml. of 0.1 mg-% standard $OD_s = 0.325$ 5 ml. of a 1 : 10 PFF
$OD_u = 0.525$
The concentration of the unknown in mg-% is

a. 0.8.
b. 1.6.
c. 3.2.
d. 9.1.

33. (034) A curve used to calculate BUN results in an example of the curve designated as

a. C-A.
b. S-A.
c. S-T.
d. nonlinear.

34. (034) If percent transmittance is plotted versus wavelength instead of O.D. versus wavelength, the resulting curve is

a. invalid.
b. skewed.
c. bell-shaped.
d. inverted.

35. (036) The best way to avoid "drift" due to significant line voltage fluctuations affecting the Coleman, Jr. Spectrophotometer is to use a

a. transformer.
b. rheostat.
c. wet battery.
d. voltmeter.

36. (036) The *first* thing you should do in performing a routine exciter lamp check on a Coleman, Jr. Spectrophotometer is to

a. set the wavelength at 400 mμ.
b. disconnect the lamp.
c. unplug the instrument.
d. clean the lamp.

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37. (040) In checking the wavelength calibration on a Coleman Jr. Spectrophotometer, you note that the galvanometer hairline is very erratic. This is probably the fault of the

a. exciter lamp.	c. power supply.
b. calibrating filter.	d. photosensitive cell.

38. (040) A flame photometer measures light which a specimen

a. transmits.	c. absorbs.
b. emits.	d. filters.

39. (042) Intensity of the characteristic light produced in flame photometry is measured by means of

a. an atomizer.	c. a burner.
b. an optical system.	d. a photosensitive detector.

40. (043) When a problem in specimen collection develops, what are the two most important considerations?

a. Time and cost.	c. Trauma and the physician.
b. Technical efficiency and time.	d. The patient and technical accuracy.

41. (044) Before blood specimens are taken for chemical analysis, fasting is

a. always required.	c. advisable if eating effects are unknown.
b. never required.	d. advisable to avoid hemolysis of the specimen.

42. (045) The danger involved in handling spinal fluid in comparison to other infectious material is

a. much less.	c. much greater.
b. about the same.	d. nonexistent.

43. (046) In addition to removing protein, a protein-free filtrate

a. eliminates pigments and reduces adverse reactions.
b. reduces adverse reactions and coagulates protein.
c. coagulates protein.
d. eliminates pigments and coagulates protein.

44. (047) What type of protein precipitation is demonstrated by the STA acid method?

a. Protein dehydration.	c. Cationic precipitants.
b. Insoluble salt formation.	d. Heavy metal precipitants.

45. (049) The objective of a good quality control program is to

a. eliminate uncertainty.	c. maintain ±1σ control.
b. check technicians' work.	d. minimize technical variation.

46. (049) A solution which has an exact known composition is called

a. control.	c. reference.
b. standard.	d. A.C.S. grade.

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47. (050-051) Which of the following statements concerning standards/controls is true?

a. A standard may be a control.
b. Standards and controls are interchangeable.
c. A control approximates the unknown specimen.
d. A control is assigned definite values.

48. (051) What word below best defines precision in quality control?

a. Reproducible.
b. Consistent.
c. Accuracy.
d. Validity.

49. (053) What statistical expression is used to describe *total* allowable variation of a test?

a. Standard error.
b. Precision.
c. Accuracy.
d. Confidence limits.

50. (053) The possibility that a control specimen will vary more than $\pm 2.5\sigma$ is

a. 32 percent.
b. 12 percent.
c. 4.5 percent.
d. 1.2 percent.

51. (054-056) What would the confidence limits of a particular test be in mg-%, if you set $\pm 2.5\sigma$ as an allowable range, $\pm 1\sigma$ is 2, and the mean control value is 100 mg-%?

a. 95 to 105.
b. 97 to 103.
c. 98 to 102.
d. 99 to 101.

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CDC 90411

MEDICAL LABORATORY TECHNICIAN - CLINICAL CHEMISTRY AND URINALYSIS

(AFSC 90470)

Volume 2

Laboratory Procedures in Clinical Chemistry (Part I)

Extension Course Institute
Air University

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Preface

ONLY GENERAL chemical and physical aspects of physiological chemistry were discussed in the first volume of this course. This and subsequent volumes will be more specific in their approach to clinical chemistry. Chapter 1, for instance, discusses specific electrolytes, blood, gases, and pH. Liver function with its associated tests and "proteins" are covered in Chapters 2 and 3. Carbohydrate chemistry, primarily glucose, is developed in Chapter 4. The final chapter is devoted to one of the most difficult analytical areas of the clinical laboratory, enzymes.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to: Med Svc Sch (MSSTW/120), Sheppard AFB TX 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB, Alabama 36114, preferably on ECI Form 17, Student Request for Assistance.

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Figure 13, taken from "Autoanalyzer N Method File, N-2a" published by Technicon Instruments Corporation, Chauncey, New York.

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Body Metabolites

ANY COMPONENT of metabolism is a *metabolite*. Included are vitamins, minerals, electrolytes, carbon dioxide, proteins, and water. Our selected use of the term "metabolite" is arbitrary, designed primarily for convenience in discussing body chemistry from a laboratory viewpoint. Electrolytes are given special consideration because of their prominence in laboratory studies and their ionic characteristics. We will consider both anions and cations, which are negatively and positively charged ions respectively. To sustain life, anions and cations must maintain an equilibrium in various parts of the body. This is a dynamic equilibrium, which results in characteristic intracellular and extracellular ion concentrations. Further, the concentration of a particular ion varies with the type of cell and area of the body. Major selective forces are described in the concepts of Donnan equilibrium and osmotic equilibrium, although other things must also be considered. In addition to equilibrium concepts, attention is devoted in this chapter to blood pH. Both the physiological and technical aspects of blood pH measurement are mentioned. Finally, the direct clinical importance of calcium and phosphorus is explored apart from the discussion of electrolytes. Proteins are treated in a separate chapter. The purpose of presenting some clinical information in our discussion is to familiarize you with the importance of these chemical entities in the body. If you know the meaning of a test, you will find it increases your interest in your work, which in turn results in careful and accurate analyses.

1. Body Electrolytes

1-1. Body electrolytes are considered at some length in this chapter because of their vital role in evaluating patient progress. You will be called upon in almost every major medical and surgical case to render data concerning a patient's electrolytes. With this information the physician regulates and restores the balance of electrolytes which is critical to the well-being of his patient. A variation of 0.5 blood pH can easily cause death. Consider each electrolyte, its normal value, and its physiological significance as you read the first section of this chapter. Also, consider how a balance is maintained by the blood buffer systems.

1-2. **Nature of Electrolytes.** A substance that conducts electricity in solution may be classified as an electrolyte. Electrolytes conduct an electric current because they dissociate in solution to produce a significant number of ions. Strong electrolytes are those that are highly dissociated. Sodium chloride furnishes large numbers of sodium and chloride ions in solution and is regarded as a strong electrolyte. As you would expect, a solution of sodium chloride is an excellent conductor of electricity. Weak electrolytes are only slightly dissociated in solution and are poor conductors. Carbohydrates such as sucrose, glucose, etc., are often presented as examples of nonelectrolytes and do not dissociate appreciably; and, hence, do not conduct electricity.

- Positively charged ions are called *cations* because they migrate to the negative pole or cathode.
- Negative ions are called *anions* because they migrate to the positive pole or anode when electrodes having an applied potential are immersed in the solution.

The cations most frequently measured in the clinical laboratory are sodium and potassium. The anions most commonly measured are chloride and bicarbonate. The electrolytes present in serum are shown graphically in figure 1. Electrolytes function in many ways to sustain the life processes. They are important in maintaining acid-base balance and controlling the pH of the blood within the relatively narrow range of 7.35 to 7.45. They are essential to cell permeability and nerve impulse conductivity as well as in regulating osmotic pressure. There would be practically no limit in describing the importance of electrolytes, and it, therefore, becomes more meaningful to discuss individual electrolytes.

1-3. In interpreting results, the clinician considers two major aspects which are normally be-

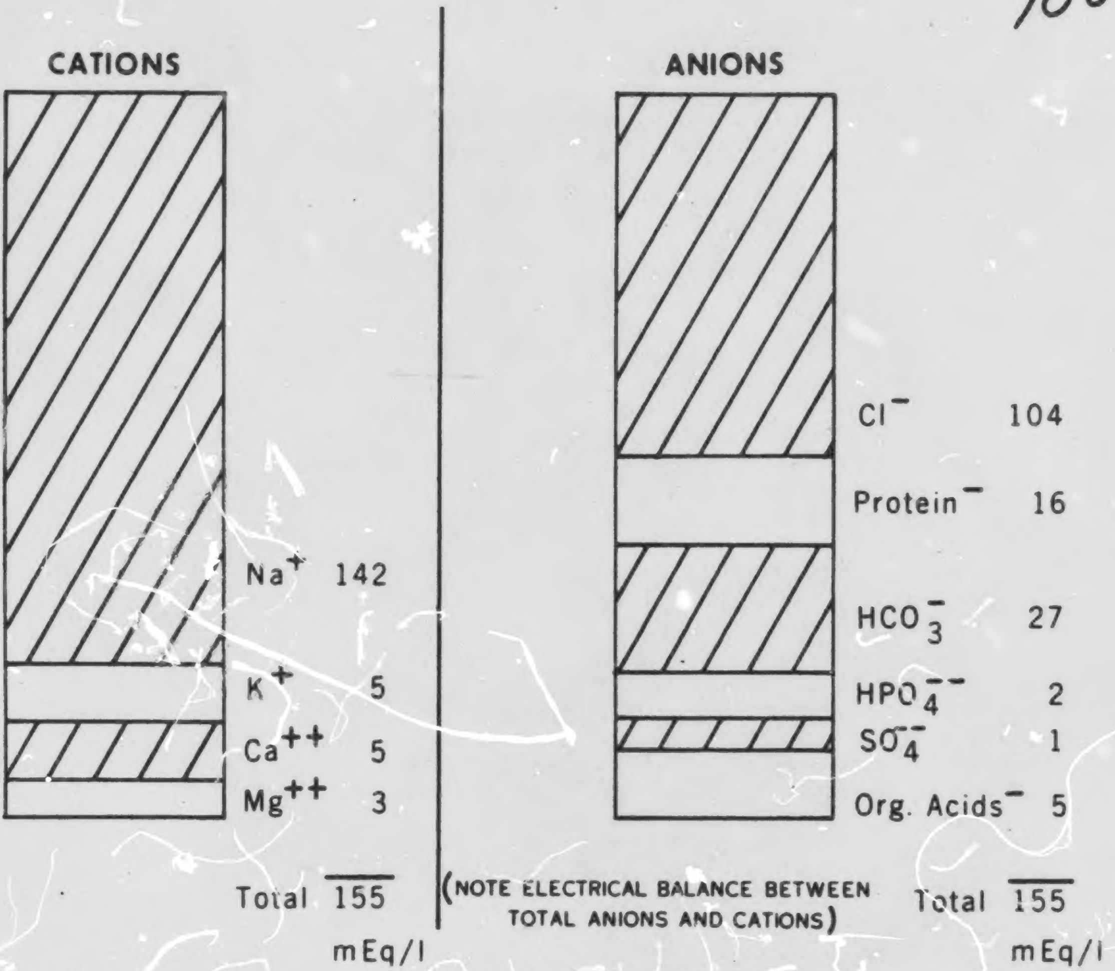

Figure 1. Electrolyte composition of serum.

yond the scope of the clinical laboratory. They are, first of all, the relationship between a particular electrolyte and the organ or system involved. For example, the chloride level would bear a direct relationship to the stomach or pancreas. Second, electrolytes are considered in relationship to each other. For this reason, electrolytes are ordered in groups commonly referred to as electrolyte batteries. Some knowledge of the relationship among electrolytes is helpful to the technician in achieving results which make sense. In this regard, there is also an element of danger: You should not allow your knowledge (or lack of it) to influence the results you report. In other words, you would not perform a potassium determination with the conscious or unconscious view of arriving at a logical balance with other results. What is logical in the laboratory may not be logical to the physician who is familiar with the clinical condition of the patient.

1-4. **Distribution of Electrolytes.** Electrolytes are not present in equal concentration throughout the body. The various concentrations differ according to whether they are intracellular or extracellular and the kind of tissue involved. This is because cell membranes are selective and only semipermeable. Further, the total of electrical charges within the cell must essentially balance the extracellular charges. Some constituents, such as proteins, do not move freely through cell membranes, but they do possess a charge and are to be considered in ionic equilibrium. The unequal distribution of ions between two areas separated by a semipermeable membrane is established by *Gibbs-Donnan equilibrium.* Without a detailed account of how equilibrium is achieved, we can state that ions shift between the plasma and the cell to maintain electrical neutrality or a slight potential difference. The equilibrium system is composed of both diffusible and nondiffusible components. The dynamic character of cell membranes[1] accounts for various biochemical exchanges between the cell and extracellular fluid. Because it is more practical to measure extracellular ion levels than ions within the cells, most of your work is with serum or plasma.

1-5. Another important aspect in the exchange of materials across a cell membrane is the process of *osmosis*. Osmosis refers to the passage of a solvent through a membrane, more significantly water through a cell membrane. Water passes from a region of lower concentration to an area of higher ion concentration. The resultant physical force exerted in a given area is termed *osmotic pressure*. Keep in mind that osmotic pressure is due to many ions present within and without the cell. Electrolytes commonly measured in the clinical laboratory are a significant part of the ions which maintain functional integrity of the cell. In most, if not all cases, you will be concerned with the fluid surrounding blood cells, though our discussion applies equally to blood cells and other body (somatic) cells.

1-6. In addition to assaying serum and plasma, you can secure more valuable data indirectly by measuring the concentration of ions in urine, spinal fluid, and perspiration. Kidneys normally conserve water and electrolytes otherwise lost and are the major means of excreting excesses. Uncompensated loss of water from the body results in dehydration, a condition which may have a profound effect upon ionic concentration. This occurs in many situations including sustained febrile conditions, diarrhea, inadequate fluid intake, and many other clinical manifestations. The decrease in fluid volume is primarily extracellular. This is accompanied by a corresponding fall in blood pressure which may lead to a state of shock. Transfer of water into the cells takes place when the concentration of ions within the cell exceeds extracellular water concentration and is due to the osmotic pressure differential. However, if the loss of water causes the extracellular fluid to become hypertonic, fluid will leave the cells. Both situations occur clinically, the latter being more common.

1-7. It is essential for you to realize that electrolyte concentration by itself says nothing of fluid volume, and hence, does not provide accurate information about total electrolyte gain or loss. Sodium and potassium may be lost from the body along with large quantities of water without any appreciable effect on electrolyte concentration, since concentration depends upon both amount of solute and volume of solvent, in this case blood volume. In most cases, the clinician is able to deduce the true extent of the deficit from both laboratory and clinical data, which emphasizes the need to interpret laboratory results in context.

1-8. **Sodium.** The concentration of sodium in plasma exceeds the concentration of any other cation. Actually, sodium comprises more than 90 percent of all the cations. The normal range is 138 to 146 mEq/L. The daily dietary intake of sodium is usually substantial. That which is not needed is excreted in the urine and to a lesser extent in perspiration. Most of the body sodium is present in fluids, but large amounts are found in the skeletal system. It is more common to find a decrease than to find an increase in serum sodium. As stated previously, there may be a loss of sodium with proportional loss of fluid, thus causing little change in the concentration of sodium. Conversely, water retention can hide the fact that total body sodium may have increased. Both sodium and potassium are measured with the flame photometer and reported in milliequivalent terms. Sodium concentration is often evaluated with respect to the concentration of bicarbonate and chloride ions. Sodium has a primary function in osmotic equilibrium, cell permeability, and muscle irritability.

1-9. **Potassium.** The normal serum potassium level in the body is 3.8 to 5.0 mEq/L. However, most of the potassium is present within body cells, with some in the extracellular fluid. It is for this reason that you should not perform potassium determinations on hemolyzed serum. The result would inevitably be higher whenever serum is hemolyzed. Elevated plasma potassium (hyperkalemia) is associated with disease of the heart and central nervous system. Regulating potassium levels is a problem for the physician in renal failure, Addison's disease, and dehydration. A low-serum potassium is frequently a postoperative problem because the patient most likely has received fluids low in potassium. The physician frequently orders postoperative potassium levels. Low-serum potassium levels (hypokalemia) may also occur in various other conditions, especially diarrhea. Potassium deficiency results in cell damage, particularly cells of the kidney tubules. We frequently associate potassium with muscle activity, nerve impulses, and, of course, acid-base balance. Like sodium, potassium functions in maintaining osmotic balance between the cells and extracellular fluid.

1-10. **Chloride.** The chloride content of plasma is not equal to the sodium concentration. This is best explained in terms of the Gibbs-Donnan equilibrium previously mentioned. In the presence of negatively charged protein, there must necessarily be more sodium ions and fewer chloride ions to maintain electrical neutrality. In more general terms, in the presence of a nondiffusible protein anion, the concentration of the diffusible sodium cation would be higher than that of the diffusible chloride anion. It should also be kept in mind that total ionic concentration within the cell is not identical with the extracellular ionic concentration, and as a result, there is a difference in osmotic pressure. As you know, chloride is usually reported based on the milliequivalent weight of sodium chloride. It would appear that little justification exists for expressing the chloride concentration in terms of sodium chloride, since the sodium concentration of plasma does not equal chloride concentration. In reality, the report to the physician reflects the same relative situation whether chloride content is reported in terms of the milliequivalent weight of chloride or of sodium chloride.

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[1] Cell membrane—it is understood this is not a membrane in the usual sense, but a selective molecular arrangement which separates the cell from its environment.

1-11. It may sometimes be difficult for you to think in terms of ions rather than compounds, and you should realize that some current laboratory practice is a carryover from days of less enlightened theory. You can easily recognize how chloride came to be reported as NaCl because sodium and chloride ions are closely associated. Most chloride taken into the body is in the form of sodium chloride, and a deficiency of one is likely accompanied by a deficiency of the other. A decreased plasma chloride develops in excessive perspiration and conditions accompanied by a decrease in plasma sodium. However, the relationship of sodium to chloride is not always simple. Situations do occur in which chloride deficiency is more striking than loss of sodium. This is likely in diminished production or malabsorption of gastric juice for the obvious reason that chloride ions are abundant in gastric juice. When chloride loss is greater than sodium loss, there will be an elevation of the serum bicarbonate to maintain electrolyte balance. The basic condition which then results is hypochloremic alkalosis. When sodium loss is greater than chloride loss, as in obstruction of the lower portion of the small intestine, there is a decrease in serum bicarbonate which results in acidosis.

1-12. **Determination of Chloride.** There are a variety of titrimetric methods to determine the concentration of chloride ions in body fluids. They are commonly performed on serum, spinal fluid, perspiration, and sometimes on urine. The procedure most widely accepted in Air Force facilities is that of Schales and Schales. A sample is titrated with a solution of mercuric nitrate. In the presence of S-diphenylcarbazone, after all mercuric ions have united with the chloride ions to form ionized mercuric chloride, the excess mercuric ions will produce a lavender color. If serum is titrated directly without preparation of a PFF, the pH is sometimes high enough to produce a purple color immediately upon addition of mercuric nitrate. To prevent confusion, you may add a drop or two of 0.1N HNO_3 or continue to add mercuric nitrate quantitatively until the color disappears. As discussed in a previous volume, we recommend that you prepare a protein-free filtrate to prevent binding of mercuric ions with the protein. The normal value for a serum or plasma chloride is in the range of 98 to 106 mEq/L. The normal value for spinal fluid chloride is 123 to 128 mEq/L, notably higher than the normal for serum. For reasons of consistency with other electrolyte values, chloride is not usually reported in mg-%.

1-13. Another method of measuring chloride makes use of silver compounds. The technique of Volhard, introduced just before the turn of the century, uses silver nitrate. After precipitation, the excess silver ions are titrated with a thiocyanate. A more recent procedure using silver compounds is that of Sendroy, which was introduced in 1937. In the Sendroy procedure, the chloride ion is reacted with silver iodate ($AgIO_3$), resulting in the formation of insoluble silver chloride and iodate ions. After the solution is filtered or centrifuged to remove AgCl, the iodate ion concentration is determined as an index of chloride present before the reaction. The iodate ions are measured in various ways, usually by a thiosulfate-starch titration. This procedure can be used with accuracy, but is not quite as simple as the Schales and Schales method.

1-14. Sweat electrolyte determinations are essential for the early diagnosis and treatment of children with cystic fibrosis. This disease is one of the most serious chronic diseases of childhood.[2] Sweat chloride is most often used as a screening test since in cystic fibrosis these chloride values range above 60 mEq/L, while in normal children the concentration does not exceed 60 mEq/L. Heterozygous individuals are reported to range from 40 to 60 mEq/L in sweat chloride concentration. Porous paper and agar, impregnated with reactant chemicals, have been used as a simplified sweat chloride screening test. However, the literature is not in agreement on the efficacy of this method.

1-15. The problem of obtaining a sufficient quantity of sweat, without undue stress to the child, has been solved with the introduction of pilocarpine iontophoresis to induce localized sweating. The procedure must, of course, be supervised by a physician. A simple iontophoresis instrument, described in the *Medical Service Digest* of December 1965, costs less than $10.00 to build. A slight electrical charge from the iontophoresis instrument causes pilocarpine to enter the skin tissue. The area (forearm or leg) is thoroughly washed and rinsed before chemically clean gauze or porous paper is placed to absorb the sweat. Ultramicro titrimetric (Schales & Schales) or electrometric determinations for chloride have been used directly on sweat obtained by centrifugation. Usually, a sufficient amount is recovered to also determine the sodium concentration on a diluted specimen.

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[2] Dubowski, K.M., "Some Practical Simplifications of Perspiration Electrolyte Analysis (Sweat Test)," *Clinical Chemistry*, Vol. 7, #5, 1961.

1-16. **Bicarbonate and Carbon Dioxide Content.** No electrolyte battery is complete without a determination of carbon dioxide content. Some instruments measure carbon dioxide volumetrically; others may be manometric, as shown in figure 2. Subsequent discussion will show you the difference in interpretation of CO_2 content and CO_2 combining power. Another technique measures the plasma or serum bicarbonate level by titration with a standard acid and back-titration with a standard base. These manometric, volumetric, and titrimetric procedures are published in detail in AFM 160–49, 1 April 1967 Revision, *Laboratory Procedures in Clinical Chemistry*. All three procedures have been used extensively in hospital laboratories. More recently an automated colorimetric method employing earlier titrimetric principles has been introduced for the Autoanalyzer.[3]

1-17. The fundamental purpose of measuring CO_2 content is to enable the physician to evaluate buffering capacity of the blood to maintain a pH of 7.4. Relatively little carbon dioxide is normally present in the plasma as dissolved CO_2. Instead, it is carried in the cells and plasma in combination with hemoglobin and other proteins in the form of bicarbonate, and a small amount as carbonic acid. To maintain a pH of 7.4, a ratio of 20:1 must be maintained between bicarbonate and carbonic acid. (There are also other buffer systems in the body that are not discussed here.) Before studying the Henderson-Hasselbalch equation, you should review the concept of ionization in Volume 1 and "pH" in Chapter 3 of volume 1.

1-18. You will remember that pH may be expressed as a negative logarithm of the hydrogen ion concentration, $pH = -\log [H^+]$. At a pH 7.4, then, the $[H^+]$ is $10^{-7.4}$ moles per liter. The equilibrium expression for ionization of any weak acid, such as H_2CO_3, in aqueous solution was detailed in Volume 1. This ionization expression for carbonic acid is

$$H_2CO_3 = H^+ + HCO_3^-$$

where H_2CO_3 is the acid; H^+, the hydrogen ions; and HCO_3^-, the bicarbonate ions. The ionization constant (K_a) is the ratio of the product of hydrogen and bicarbonate ion concentrations to the concentration of carbonic acid, or mathematically

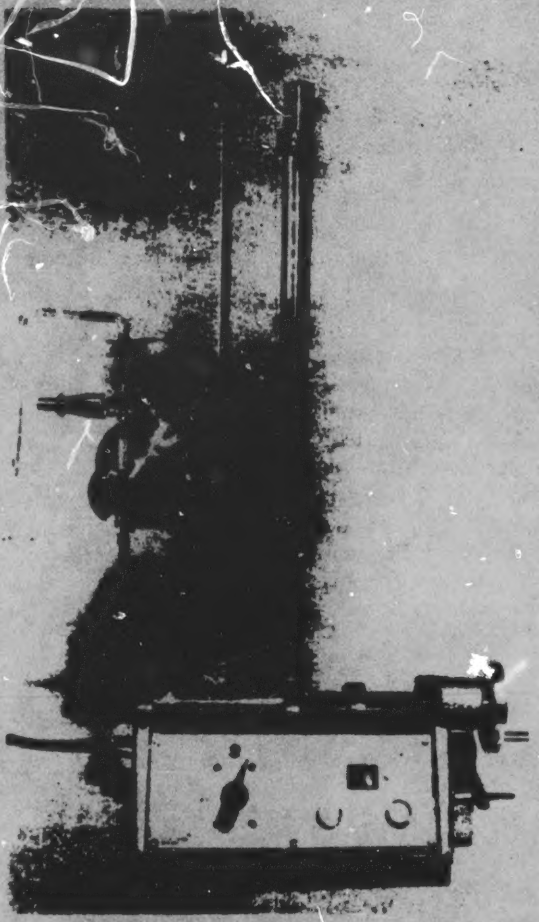

Figure 2. Natelson microgasometer for the manometric determination of carbon dioxide.

$$K_a = \frac{[H^+]\ [HCO_3^-]}{[H_2CO_3]}$$

It is more convenient to express this in its logarithmic form ($-\log K_a$) when making calculations with pH. Introducing the definition of $pH = -\log [H^+] = 10^{-pH}$, therefore, $pK_a = -\log K_a = 10^{-K_a}$. The pK_a for this reaction in blood is dependent upon temperature, ionic strength, and the presence of protein.

1-19. In terms of the Henderson-Hasselbalch equation for buffers, the carbon dioxide buffering system is expressed as follows:

$$pH = pK_1 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \qquad (1)$$

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[3] Autoanalyzer is the registered trademark of Technicon Corporation.

The pK value for this buffer system is 6.1. Using this value, we could write the equation as follows:

$$10^{-7.4} = 10^{-6.1}\,\frac{[HCO_3^-]}{[H_2CO_3]}$$

Then solve for the ratio as follows (see footnote 4):

$$\frac{[HCO_3^-]}{[H_2CO_3]} = \frac{10^{-7.4}}{10^{-6.1}} = \frac{1}{10^{-1.3}} = \frac{1}{20}$$

You will find that the pK_1 value in (1) above is not the pK_a of carbonic acid. Rather, it is the pK for dissolved CO_2, which is proportional to carbonic acid concentration.

1-20. The partial pressure (pCO_2) of carbon dioxide in the lungs will determine the amount of dissolved CO_2 and small amount of carbonic acid that will be in equilibrium in the plasma. This relationship is expressed mathematically as:

$$\begin{matrix} H_2CO_3\ \text{mM/L} \\ \text{or} \\ CO_2\ \text{dissolved} \end{matrix} = a \cdot pCO_2 \tag{2}$$

where "a" is a constant known to be 0.03 for dissolved CO_2 in mM/L. It follows that pCO_2 is proportional to carbonic acid or dissolved CO_2 concentration.

1-21. Carbon dioxide will diffuse to any other phase (solid, liquid, or gas) which has a lower partial pressure and is exposed to the plasma. This is the reason why blood specimens for CO_2 content and direct pH measurements *must be drawn anaerobically*. This can be done in a sealed vacuum tube system or capillary tube if the container is filled completely to minimize a gas (air) phase in contact with the blood specimen. In the past, blood was collected anaerobically under oil but this method has not been used generally since the advent of suitable vacuum tubes. Heparin is the anticoagulant of choice for this collection. The equation in (2) may be substituted in the Henderson-Hasselbalch equation (1). Thus:

$$pH = pK_1 + \log \frac{HCO_3}{a \cdot pCO_2} \tag{3}$$

CO_2 content (total CO_2) includes both bicarbonate and CO_2 dissolved (carbonic acid, H_2CO_3),

$$CO_2\ \text{content} = HCO_3 + CO_2\ \text{dissolved}$$

Substituting equation (2) for carbon dioxide dissolved:

$$\begin{gathered} CO_2\ \text{content} = HCO_3^- + (a \cdot pCO_2) \\ \text{and} \\ HCO_3^- = CO_2\ \text{content} - (a \cdot pCO_2) \end{gathered}$$

Substituting this in the Henderson-Hasselbalch equation (1),

$$pH = pK_1 + \log \frac{[CO_2\ \text{content} - (a \cdot pCO_2)]}{a \cdot pCO_2} \tag{4}$$

This formula describes the relationship of carbon dioxide partial pressure (pCO_2) to acid-base balance as it is expressed in the Henderson-Hasselbalch equation. The significance becomes more apparent when you realize that this acid-base relationshp can be defined if either the pH or CO_2 content is determined at a known pCO_2.

1-22. This concept is the basis for a relatively new approach to quantitation of the factors involved in acid-base balance as proposed by Astrup in 1961.[3] The Astrup approach uses a capillary glass electrode to measure blood pH at two known pCO_2 values, one high and one low. Whole blood is equilibrated with each of these gases at 38° C. before the pH is measured. These two points are plotted on a prepared nomogram with pH versus pCO_2, as shown by points A and B in figure 3. When these two points are joined by a straight line, all relevant acid-base data can be obtained from the nomogram. When the pH of an anaerobic blood specimen is determined, the actual pCO_2 is obtained from the point of intersect with the straight line plotted previously. This is indicated by point F and the dotted lines in figure 3. The actual pCO_2 and pH values of the patient may now be substituted in the Henderson-Hasselbalch equation (4) to establish the state of acid-base equilibrium.

1-23. Astrup introduced several new terms which may be calculated from his nomogram. They are defined as:

Standard bicarbonate—the bicarbonate concentration of plasma from completely oxygenated blood equilibrated at 38° C. and a pCO_2 of 40-mm Hg.

Base excess—the excess (positive value) or deficit (negative value) of base in mEq/L, assuming normal base excess is zero at pH 7.38, and a pCO_2 of 40-mm Hg on completely oxygenated blood.

Buffer base—the sum of all buffer anions (mEq/L) in 1 liter of blood.

The arrangement of these values can be seen on the nomogram in figure 3. Several American-made pCO_2 instruments are available with a direct readout of pCO_2 and nomograms for other calculations in acid-base balance.

1-24. Let us summarize what the previous discussion of acid-base balance means to you as a laboratory technician. First, it is obvious that

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[4] The antilog of 1.3 is 20.

[3] Astrup, Poul. "A New Approach to Acid-Base Metabolism." *Clinical Chemistry*, 7:1, 1961, Hoeber, Inc., Harper & Brothers, New York.

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MODIFICATIONS

Figure 3 of this publication has(have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted materials are copyrighted and could not be duplicated for use in vocational and technical education.

since bicarbonate represents two-thirds of the blood-buffering capacity. an adequate measurement of bicarbonate will establish the state of acid-base balance. However the bicarbonate determination must be made with regard to clinical physiology and the Henderson-Hasselbalch equation. This equation (1) has three unknowns: pH, bicarbonate. and carbonic acid concentrations. The equation can be solved if any two of these unknowns are determined. However, this form of the equation is valid only when the pCO_2 is normal. That is when there is no pulmonary involvement or abnormal respiration. The substituted form of the Henderson-Hasselbalch equation (4) accounts for any change in pCO_2 and has three unknowns: pH, CO_2 content, and pCO_2. It is evident that either pCO_2 or pH must be determined in addition to CO_2 content to adequately solve this equation.[5,6,7,8,9,10]

1-25. In conclusion you should note that CO_2 combining power has been excluded from this discussion of acid-base balance. In 1932 Van Slyke recognized the limitations of CO_2 combining power for evaluation of blood-buffering capacity. Since the titrimetric method for bicarbonate (paragraph 1-16) also disregards the physiological significance of pCO_2, it is for all practical purposes the same as CO_2 combining power. Both of these tests are of limited value in assessing acid-base equilibrium and may, in fact, be entirely misleading.

1-26. Although most of the buffered carbon dioxide is transported in the plasma as bicarbonate, more than three-fourths of the total carbon dioxide is carried by the red blood cells. This can be explained in terms of ionic shifts which take place between the red blood cells and the plasma. As bicarbonate (HCO_3^-) moves from the red blood cells into the plasma, chloride ions move into the cells to maintain electrical neutrality. You may recall there are primarily potassium ions within the cells and sodium ions in the plasma to balance the anions present. The movement of chloride to replace bicarbonate in balancing potassium is referred to as the *chloride shift*. The entire problem is one of preserving the blood pH while the patient may encounter either respiratory or metabolic activity that tends to change the ratio or carbonic acid to bicarbonate. The various conditions which may occur are respiratory acidosis or alkalosis and metabolic acidosis or alkalosis. Each condition characteristically affects the ratio and may be compensated by various factors. In metabolic acidosis there is a decrease in bicarbonate. This may occur in renal disease or in diabetes mellitus. Diabetic acidosis results from loss of bicarbonate and increase of carbonic acid following the release of hydrogen ions from the breakdown of fats and proteins (gluconeogenesis). The opposite is encountered in metabolic alkalosis, wherein there is little or no change in carbonic acid content of the blood. This may occur as a result of intestinal obstruction, in which case the chloride may also decrease. In certain respiratory diseases there is an increase in carbonic acid relative to bicarbonate, and the effect is respiratory acidosis. Finally, in respiratory alkalosis, the carbonic acid decreases with a disproportionate decrease in the bicarbonate. The chloride may be elevated as well as in hyperventilation. Knowledge of the plasma CO_2 content and pCO_2 or pH aids the clinician immeasurably in evaluating symptoms relating to the treatment of his patient.

1-27. **Electrometric Measurement of pH.** The physician does not always have a clinical history or prior knowledge of a patient's disorder. Lacking information on the etiology of a patient's condition, the physician would not necessarily know if the diagnosis should be acidosis or alkalosis.

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[6] Nadeau. G., "A Simple Method for Combined Determination of Plasma Bicarbonate. pH. and Chloride." *American Journal of Clinical Pathology*. 23:710-712. July 1953. Williams and Wilkins Co.. Baltimore.

[7] Hodes. M.E.. "CO_2 Content (Titrimetric)." *Standard Methods of Clinical Chemistry*. Vol. 1. Reiner. Miriam (ed.). 1953. Academic Press. Inc.. New York.

[8] Henry. Richard J.. *Clinical Chemistry Principles and Technics*. 1964. Hoeber Med. Div.. Harper & Row. New York.

[9] *Handbook of Clinical Laboratory Data*. Damm. H.C.. (ed.). 1965. The Chemical Rubber Co.. Cleveland. Ohio.

[10] Miller. Seward. *A Textbook of Clinical Pathology*. 1966. Williams & Wilkins Co.. Baltimore.

Further, this cannot be determined with certainty from carbon dioxide levels alone. It is, therefore, necessary to measure blood pH directly or to calculate pCO_2 in addition to measuring CO_2 content. Because of the narrow limits within which blood pH must be measured, only specially designed pH meters are sensitive enough for this purpose. Even in diabetic coma, the pH of the patient's blood may not vary from the normal by more than 0.5 pH unit. There are pH meters available today which can measure to $\pm$ 0.001 pH unit on samples as small as 0.015 ml.

1-28. Regardless of design or sensitivity, pH meters all measure electromotive forces. This cannot be accomplished with a single electrode, because potential difference exists between two electrodes. With one of the electrodes as a reference electrode, the potential of the other is measured in comparison with this standard. Various electrodes are in use—five are discussed here. The first is a hydrogen electrode, which is arbitrarily assigned a value of 0.0 volts. It is composed of a strip of platinum which has been coated with platinum black. When this electrode comes in contact with hydrogen gas and water, electrons are released and adhere to the metal. The electrode will then have a negative charge. Hydrogen electrodes are prepared in which the hydrogen gas is under a pressure of one atmosphere. If the temperature is held constant, the electrode will maintain a standard value. This type of electrode is *not* used for blood because the carbon dioxide would be removed by the hydrogen. The hydrogen electrode is the standard electrode upon which all others are based.

1-29. A second type of electrode is the calomel electrode. This electrode is usually the reference electrode used in blood pH measurements. Calomel electrodes are less difficult to prepare and maintain than hydrogen electrodes. They are composed of mercury, a paste of Hg_2Cl_2, and a solution of potassium chloride (KCl). Under standard conditions a saturated calomel electrode [illegible] have a potential of 0.246 volts.

1-30. A third type of electrode used is the quinhydrone electrode. It is used in solutions in which hydrogen of the hydrogen electrode would react with a component of the solution. The rationale behind this type of electrode is that hydroquinone ($C_6H_4(OH)_2$) reacts with water to yield quinone ($C_6H_4O_2$) and hydronium ions with the release of electrons.

1-31. A fourth type of electrode is the glass electrode, which consists of a metal such as platinum immersed in an acid solution and inclosed in permeable glass. This is the blood pH electrode. The principle of the glass electrode is quite involved, but essential characteristics are established by a difference in hydronium ion concentration on either side of a glass membrane. For this reason, most of the older glass electrodes on the market are not valid for solutions of high pH or very low pH, since what passes through the glass membrane is affected by extremes in pH.

1-32. The fifth electrode we mention is composed of a rod of antimony coated with antimonous oxide. While this electrode is not usually used in the U.S. Air Force, you should be aware of its existence.

1-33. Regardless of the type of electrode, pH meters measure potential difference between two electrodes, one of which is standardized. Temperature affects the determination and must be controlled whenever pH is measured within narrow limits. Potential of the circuit established is measured with a potentiometer after amplification. Only when the cell resistance exceeds 1 megohm is a special voltmeter necessary. This is the case when glass electrodes are a part of the system. Circuit diagrams for particular pH meters can be obtained from the manufacturer of the particular instrument. It is sufficient for you, as a technician, to understand how it is possible to determine hydrogen ion concentration electrically. Models vary widely in mechanical design and in the adjustments they require. Some are equipped with thermocompensators which eliminate the necessity for manual temperature adjustments.

1-34. Standardization is always necessary, and is accomplished with one or more known buffers. The most common problem in standardizing a pH meter arises when you do not know the correct pH value of your standard buffer. A series of buffers should be kept on hand, and some of these should be procured from a commercial source. Also, you should give special attention to conditions under which the buffers are stored. Contamination is always a threat to reliability, especially when several people have access to standard buffers. Finally, a pH meter should be used to measure the pH of solutions for which the instrument and electrodes were designed. As mentioned earlier, all instruments are not suitable for the measurement of blood pH. A blood pH meter, as shown in figure 4, is a highly specialized instrument.

2. Other Inorganic Metabolites

2-1. Two very commonly assayed inorganic ions are calcium and phosphorus. They are frequently requested in the clinical evaluation of metabolic disorders. As you read, try to appreciate the relationship between calcium and phosphorus levels.

2-2. **Calcium.** The most abundant cation of the body is calcium because it makes up a large part of the skeletal system. Calcium is also present in the form of calcium ions, which are present

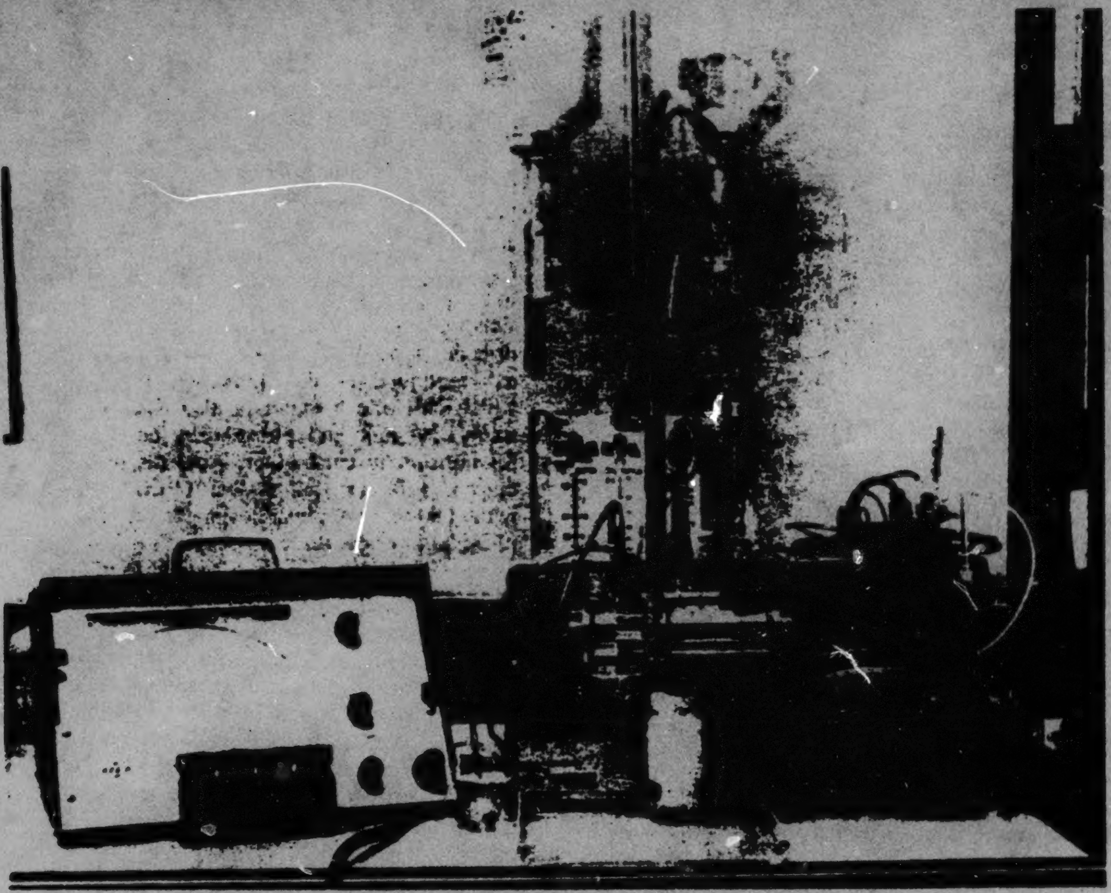

Figure 4. Blood pH meter.

in nerve and muscle tissue as well as in the coagulation mechanism of blood. It may be recalled that many of the anticoagulants added to blood specimens function by precipitation of calcium. One does not normally encounter a calcium deficiency which is severe enough to interfere with the body's coagulation mechanism.

2-3. The normal intake of calcium amounts to several grams per day, with an essential daily requirement of just over 1.5 Gm. Various factors facilitate the absorption of calcium, including pH of the intestine and the presence of Vitamin D. The normal serum level is 4.5 to 5.5 mEq/L or 9 to 11 mg-%, with no reported variation related to diet or fasting condition of the patient. Blood cells, themselves, contain a negligible amount of calcium. Besides being in the form of ions, calcium in the nondiffusible form combines with certain plasma constituents. Clinically, a decrease in ionizable calcium is associated with tetany, a condition characterized by spasms of the extremities. Tetany may occur during pregnancy because of the demand for calcium, especially due to lactation. There are other causes for decreased calcium, including removal or deficiency of the parathyroid glands, alkalosis, and Vitamin D deficiency. The influence of the parathyroid glands is profound. In hyperparathyroidism, serum calcium may be elevated to 20 mg-% or more. The level of calcium is invariably closely associated with phosphorus levels of the serum, and these two elements tend to maintain a constant ratio. There is usually a concomitant increase in serum phosphate levels as there is a decrease in calcium values. However, there may be deficiency of both calcium and phosphorus, as in rickets.

2-4. **Determining Calcium Levels.** There are many techniques for determining serum calcium levels. In some methods calcium is precipitated with some anion like oxalate, with quantitative estimation of the anion. Other methods titrate directly, using a suitable indicator such as ammonium purpurate. A number of serum constituents interfere with direction titration and, in addition, end points are not usually clear enough to establish this as a method of choice. Calcium can, of course, be determined directly with a flame photometer. This appears to be one of the simplest and most convenient methods, but is rejected by some laboratories on the basis of technical difficulty in achieving desired accuracy. Not all flame photometers are suitable for the determination of calcium because sodium and potassium both affect the emission spectrum of calcium by positive interference. Utilization of the photometer for Ca^{++} levels requires the use of a blank[11] containing sufficient levels of Na^{+} to overcome the interference of this electrolyte. A calcium standard is available through supply channels. Phosphate and sulfate, on the other hand, inhibit the emission spectrum of calcium. Most precise results are obtained by using an organic solvent together with a nonionic wetting agent. In any case, if a particular laboratory achieves results within the accepted standard deviation limits with the photometer it uses, there appears to be no objection to the photometric method.

2-5. It is not possible to discuss in detail all of the various techniques available for the assay of calcium. Perhaps the most widely accepted chemical method in U.S. Air Force facilities is an adaptation of the Ferro and Ham method. In this method, calcium is precipitated as the insoluble salt of chloranilic acid. It is washed free of excess chloranilic acid, using isopropyl alcohol. The precipitate of calcium chloranilate is then dissolved in a solution of ethylenediaminetetraacetic acid (tetrasodium salt). The resulting pink solution is compared photometrically against a similarly prepared standard. Where urine is used as the specimen, either the flame photometer method or the Sulkowitch determination is normally used. In the Sulkowitch test, calcium is precipitated with oxalic acid reagent and the degree of turbidity is noted. Phosphates do not precipitate with calcium, but the degree of turbidity may be affected by other urinary constituents. There is some question as to the value of such an approximation, and it is generally interpreted rather liberally.

2-6. **Phosphorus.** Like calcium, most phosphorus in the body is present in the skeletal system and teeth. Phosphorus also has a key role in the liberation of energy by what is termed the "high energy phosphate bond." The hydrolysis of adenosine triphosphate (ATP) produces about 8,000 calories per molecule. In a discussion of biological energy, it is customary to represent such a high energy bond with a curved line ($\sim$), but the energy is not actually all contained in one bond. Rather, energy is released by group transfer as ATP is hydrolyzed to produce adenosine diphosphate (ADP). This is the mode of storing energy from the oxidation of fats and sugars, and in this sense phosphorus is the powerhouse of the body. In fact, phosphorylation is essential for intestinal absorption of various dietary components, particularly carbohydrates. The normal serum phosphorus level is 3.0 to 4.5 mg-%; however, with infants to 1 year of age, it may go up to 6.0 mg-%.

2-7. As previously indicated, there is a relationship between calcium and phosphorus in the body. As calcium levels rise, phosphorus levels normally decrease. In diabetic acidosis the inorganic phosphorus level of the blood increases, but phosphates may be administered by the physician to correct a deficiency of phosphate. Inorganic phosphorus is sometimes low in rickets, as mentioned in the discussion of calcium, in which case the calcium is also decreased. Ordinarily, calcium and phosphorus bear an inverse relationship to each other. Acidosis of renal diabetes is different from acidosis of diabetes mellitus with respect to phosphorus. In renal disease, phosphate retention contributes to acidosis. To extend the analogy, in hyperparathyroidism there is a decrease in phosphorus as calcium increases. (This was also mentioned in our discussion of calcium.) It is difficult to discuss calcium and phophorus separately because levels of phosphorus necessarily relate to calcium levels. The discussions of clinical areas are by no means complete, but will reinforce your awareness of the need to consider each laboratory result in perspective.

2-8. **Laboratory Determination of Phosphorus.** As mentioned in the discussion of filtrates, phosphorus determinations are made using a trichloroacetic acid (TCA) filtrate. In the method of Fiske and Subbarow, outlined in AFM 160–49, phosphorus reacts to form a phosphomolybdate complex. Quantitative reduction of the complex with aminonaphtholsulfonic acid (ANSA) produces a blue color of an intensity proportional to the inorganic phosphate content. The ANSA reagent is unstable and should be prepared fresh at least every 2 weeks. Dry ANSA mixture can be secured commercially in small vials sufficient for most laboratories. TCA filtrate contains a mixture of inorganic phosphate ions as well as organic phosphorus. It is possible to measure one or both constituents, but the method described is for the inorganic phosphate. It is termed "inorganic phosphorus" instead of inorganic phosphate, because calculation is in terms of phosphorus. The Fiske and Subbarow method is an example of a category of reactions in which molybdate will react with a phosphate, silicate, or arsenate to form the molybdenum blue color. It is also the method used with the Autoanalyzer. ANSA is one of the compounds that can be used in the reduction step. Other adaptations use stannous chloride, ferrous sulfate, hydroquinone, ascorbic acid, or other reagents.

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[11] The blank most often used is "Reagent 12," which contains 600 mEq/L. sodium and 0.02 percent Sterox SE.

2-9. Another general category of methods for the determination of phosphorus involves the reaction of a molybdate with an acidified solution of orthophosphate and vanadate to produce a yellow color. This method is reported to yield results slightly higher than the Fiske and Subbarow technique. Although it has the disadvantage of deviating slightly from Beer's law, the yellow color is more stable than the blue color.

2-10. A third general method by which phosphorus is determined uses the use of a dye and is not usually performed in clinical laboratories. If you perform a urine phosphorus, you should prepare a 1/100 dilution with water, followed by the addition of 30 percent TCA to an aliquot of the diluted urine. The sample is centrifuged and the supernate must be clear. The dilution factor is considered in the calculations.

2-11. Regardless of method, there are a few problem areas in performing phosphorus determinations. It is one of the few tests which is definitely affected by a nonfasting condition of the patient. Perhaps more important is the fact that whole blood samples are not stable. Intracellular phosphates hydrolyze and may, within a few hours, increase the serum phosphorus level by 100 percent. If the cells are separated from the serum, the specimen should be stable in the refrigerator for several days. Another potential source of error is the use of cleaning compounds which contain phosphorus. The possibility of contamination should always be considered if phosphorus results are elevated.

CHAPTER 2

Liver Function Tests

ALTHOUGH FUNCTIONS of the liver are numerous and complex, certain well-defined tests in the clinical laboratory have become more or less routine as liver function tests. The most common are thymol turbidity, bilirubin, BSP, and cephalin-flocculation. Some enzyme tests are classified as liver function tests, but will be discussed in a later chapter. Cholesterol is included in this chapter because it is formed in the liver and sometimes used as a diagnostic aid in liver disease. Cholesterol is not ordinarily considered part of a battery of liver function tests. It is not suggested that tests discussed in this chapter are necessarily limited to liver functions or that the list of tests in this chapter is complete. Any laboratory test may, and frequently does, serve more than one purpose.

3. Physiological Basis of Liver Function Tests

3-1. If you have some idea how the liver functions, you will gain an insight into the purpose and basis of liver function tests. The complexity of liver functions relates to equally complex biochemistry. As a clinical chemistry technician, you are expected to know the nature of the tests you perform for liver function. This implies an elementary understanding of the liver itself.

3-2. **Anatomy and Physiology of the Hepatic System.** The liver is the largest organ of the body, with an average weight of 1500 g in the adult male. It has two main lobes and is located in the right hypochondrium extending across the epigastrium to the left hypochondrium. Locate the liver and its associated organs in figure 5. The upper portion of the liver is overlaid by the lungs (pleura) and diaphragm. The lower portion overlaps the stomach and intestines. The liver is covered by a collagenous capsule which extends along the structures which enter it. The intralobular bile duct runs between the liver lobules which make up the lobes of the liver. Connective tissue of the liver supports the hepatic portal vein, which brings blood containing food absorbed in the intestine. Besides the bile ducts and the portal vein there are also the lymphatics and the portal artery. Briefly stated, the major functions of the liver are: (1) formation of blood constituents such as prothrombin and destruction of red blood cells; (2) detoxification of harmful substances[12]; (3) metabolism; (4) secretion of substances such as bilirubin conjugates, cholesterol, dyes, etc.; (5) circulation of blood from the portal system; and (6) storage of glycogen and metabolic intermediates. The liver may be described as both an endocrine and an exocrine gland. It is an endocrine gland because it secretes useful substances into the bloodstream. (The term "endocrine" is not restricted to the secretion of hormones, although the liver does indeed secrete hormones which it receives from other glands of the body.) An exocrine secretion of the liver is, of course, bile. The liver is also antiendocrine in the sense that it removes hormones from the blood. An adult liver secretes 500 to 600 ml of bile in 24 hours. When liver tissue is destroyed, it demonstrates a remarkable capacity to regenerate. Interstitial inflammation with contraction of the liver tissue is referred to as *cirrhosis*.

3-3. The gallbladder is an organ extending from the bile duct which stores and concentrates secretions of the liver before they pass into the duodenum. The entire extrahepatic bile system is composed of hepatic ducts, the common bile duct, the cystic duct, and the gallbladder itself. The common hepatic duct is formed from a juncture of the right and left hepatic ducts and is also joined to the cystic duct. The gallbladder is attached to the lower side of the liver and is about 10 cm long and 5 cm in diameter. Histologically, the gallbladder consists of a folded mucous layer of cells, a muscular layer and two delineated serosal layers of cells. The physiological significance of bile is directly related to di-

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[12] Liver function tests are an essential part of the Air Force Preventive Medicine Program relating to industrial toxic hazards.

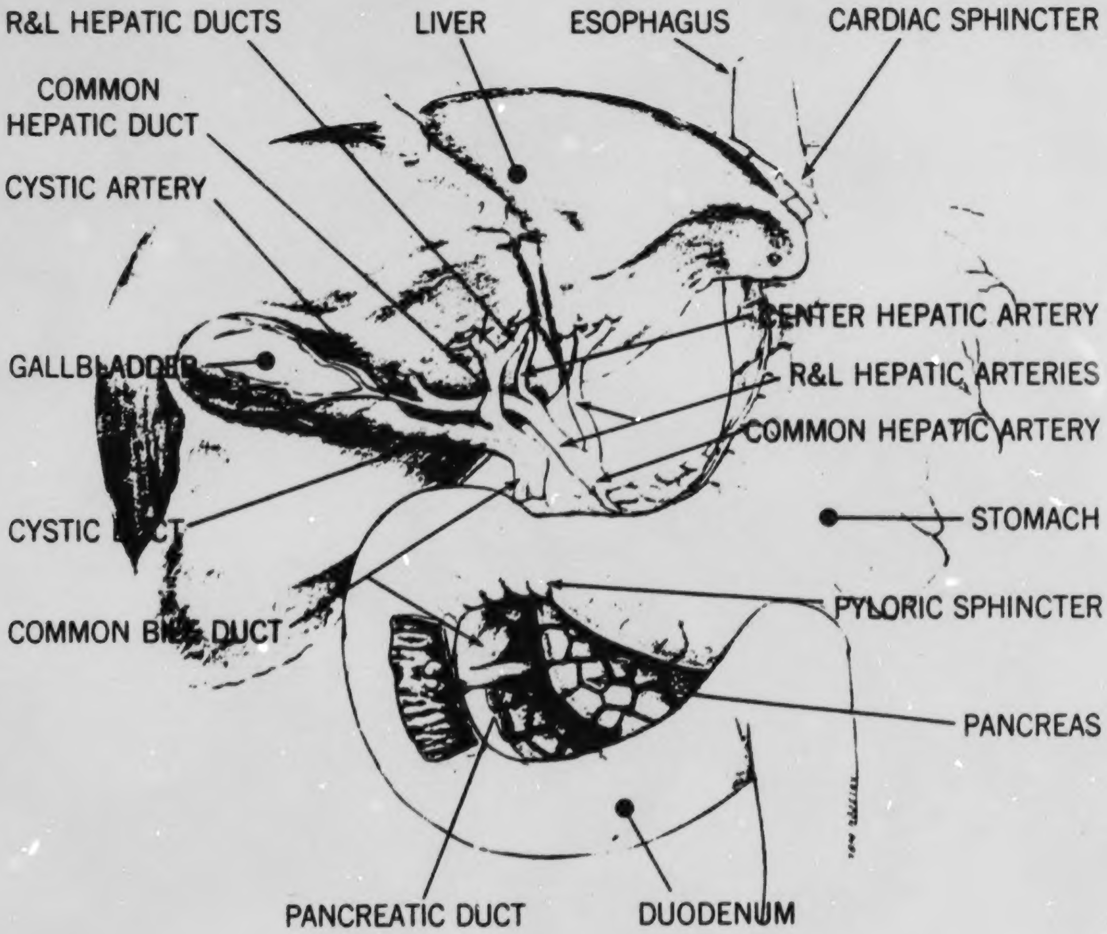

R&L HEPATIC DUCTS
LIVER
ESOPHAGUS
CARDIAC SPHINCTER
COMMON HEPATIC DUCT
CYSTIC ARTERY
CENTER HEPATIC ARTERY
GALLBLADDER
R&L HEPATIC ARTERIES
COMMON HEPATIC ARTERY
STOMACH
CYSTIC DUCT
PYLORIC SPHINCTER
COMMON BILE DUCT
PANCREAS
PANCREATIC DUCT
DUODENUM

Figure 5. The liver and associated organs.

gestion and absorption of food. It activates lipolytic and proteolytic enzymes from the intestine and pancreas and emulsifies fats. Bile also functions in an excretory capacity, particularly with regard to cholesterol which is excreted in the bile as cholic acid. The discharge of bile into the intestine is controlled by two mechanisms. The first is the nervous system and the second is activation by the hormone, cholecystokinin. Removal of the gallbladder surgically is accomplished by a procedure termed "cholecystectomy." Following surgery, the bile ducts assume some of the activity of the gallbladder.

3-4. **Bile Pigments and Related Pathology.** In the normal formation of bile pigment by the liver, the destruction of red blood cells results in the production of biliverdinglobin, which is taken up by the reticuloendothelial system as well as by the Kupffer cells of the liver. In these particular cells the formation of indirect bilirubin takes place. Upon further modification by cells of the liver, indirect bilirubin is transformed into direct-reacting bilirubin. Once the direct bilirubin passes into the biliary passages it may be oxidized to biliverdin, which is green. Finally, direct-reacting bilirubin may reach the intestines, where intestinal bacteria reduce it to urobilinogen. Urobilinogen is made up of both mesobilinogen and stercobilinogen. Bilirubin, as such, does not appear in the feces except in pathological conditions. Most of the pigment of feces is derived from the oxidation of urobilinogen into urobilin. It is noted that resorption takes place in the intestine. This includes the resorption of urobilinogen as well as other constituents from the bile. Normally, only a small amount of bilirubin is present in the blood and will not appear in the urine except in pathological conditions. A small amount of urobilinogen is released in the liver and appears in the urine.

3-5. A disturbance in the formation or flow of bile will result in *jaundice*, a syndrome characterized by pigmentation of the skin. Consider first the jaundice which is not due to an obstruction of bile flow. Overproduction of bile as manifested in hemolytic jaundice is one type of nonobstructive jaundice. Due to overproduction, there is an excess of biliverdinglobin which leads to an excess of indirect bilirubin. The direct bilirubin remains normal and no bilirubin will appear in the urine. Urobilinogen, however, is increased, and if increased sufficiently will result in an elevated urine urobilinogen. A second type of nonobstructive jaundice is called "retentive jaundice," manifested by an increase in the indirect bilirubin of the blood without an increase in urobilinogen.

3-6. Now let us consider the types of jaundice in which there is an impairment of bile flow. The obstruction may be complete or incomplete, intrahepatic or extrahepatic. In complete extrahepatic obstruction, there is a marked rise in the indirect bilirubin, with some rise in the direct-reacting bilirubin. The urine urobilinogen is negative because no bile reaches the intestine. If the obstruction is incomplete, as is frequently the case with gallstones, some bilirubin will pass into the intestine. Less urobilinogen is produced, but an increase of urine urobilinogen may occur due to maltransformation of urobilinogen by the liver. Intrahepatic obstruction is due to changes within the liver. In liver cell degeneration, serum bilirubin is high and bilirubin appears in the urine. Intestinal urobilinogen is decreased, but urine urobilinogen is increased due to maltransformation of urobilinogen in the liver.

3-7. The interpretation of laboratory findings in various types of jaundice is, of course, a clinical matter and is not within the province of the laboratory. However, you can appreciate from the preceding discussion how liver function tests can aid a diagnostician in separating forms of liver disease, such as the types of jaundice. It should be clear to you that bile pigments are all derived from the breakdown of hemoglobin and involve fairly complex chemistry. The free (indirect) bilirubin which is formed in the reticuloendothelial system is conjugated with glucuronic acid in the liver to produce direct bilirubin. The bilirubin is preceded by the formation of biliverdin. Of all the bile pigments, the clinical laboratory is primarily concerned with serum bilirubin, urobilin, and urobilinogen.

3-8. **Cholesterol.** A significant function of the liver is the production of cholesterol. It is apparently synthesized from acetate ions and later excreted principally as cholic acid. Because of the importance of cholesterol in clinical medicine, we will consider it in some detail. Cholesterol is a white crystalline substance first isolated from gallstones, which sometimes form in the gallbladder and bile passages. It is a monohydroxy alcohol with the formula $C_{27}H_{45}OH$, and one double bond, as shown in figure 6. Slightly more dense than water, cholesterol has a molecular weight of about 386 and is relatively insoluble in water. Cholesterol may also be classified as a lipid, which is a category of organic compounds soluble in fat solvents. It is a sterol, characterized by the cyclic structure of the phenanthrene ring. Cholesterol accounts for about 90 percent of the steroids of the body. Hormones are also steroids and closely related to cholesterol, although cholesterol is not a hormone.

3-9. One fundamental category of reactions which cholesterol enters into is the formation of *esters*. Esterification of cholesterol is a chemical reaction in which the OH group of cholesterol is replaced by the alkoxy group of an organic acid. About 75 percent of the cholesterol in the body is in the form of esters. Esterification in the body is catalyzed by an enzyme of the pancreatic juice called cholesterol-esterase. That which is not in the form of an ester is termed "free cholesterol." It has recently been suggested that cholesterol may exist in the serum in fractions other than free cholesterol, or esters.

3-10. The diagnostic significance of ester levels has become a matter of questionable importance in many medical facilities. Separating the free cholesterol from the ester usually involves precipitating the free cholesterol as an insoluble compound with a plant extract which is a saponin called *digitonin*. Knowledge of the free cholesterol to esterified cholesterol ratio does have diagnostic significance. The question is whether this same information can be obtained from other tests, and whether the application is sufficient to warrant routine performance of cholesterol esters analysis.

3-11. As stated earlier, total cholesterol is most significant to the clinician because of its direct relationship to liver cell damage and its more or less understood relationship to vascular diseases, such as atherosclerosis. Among the functions attributed to cholesterol are:

a. It forms a large portion of tissue structure, particularly the brain.
b. It is considered a precursor of hormones, since all adrenal hormones are derived from cholesterol.
c. Evidence suggests it can detoxify certain agents in the blood.
d. It enhances antigen activity, probably by absorption, to increase surface area.
e. Cholesterol functions in fat transport.
f. It is a constituent of bile salts that emulsify fats.

Cholesterol is not a source of energy as are fats and carbohydrates. Since cholesterol is formed in the liver, it tends to be lower in cases of liver cell damage. Cholesterol originates from the diet and by synthesis in the liver, and to a lesser extent, in other body cells.

4. Laboratory Test of Liver Function

4-1. As a competent technician, you probably feel more secure discussing specific laboratory tests than discussing their relationship to medicine. Indeed, this is where you should develop the greatest facility. The following discussion strikes at the very essence of your career field. Consider this material carefully and research the areas that are not clear and those which are of special interest to you. Liver function tests are of tremendous value in both clinical and preventive medicine.

4-2. **Bilirubin.** Bilirubin combines with diazonium salts to form azo dyes. This is the chemical basis of the Malloy and Evelyn test for serum bilirubin. Direct bilirubin can be distinguished from indirect by adding alcohol to bring out the remaining bilirubin which did not react with diazo reagent in an aqueous medium. Do you recall from your previous studies what we mean by direct, indirect, and total bilirubin? Bilirubin which reacts in 1 minute is referred to as the *direct*, whereas that which reacts in the tube after the addition of alcohol is the *total*. Total minus direct equals the *indirect*. The so-called prompt reacting bilirubin is not really synonymous with direct bilirubin. Prompt bilirubin is usually read in 15 to 30 seconds. The color does intensify with time, and therefore, the time called for in each procedure must be uniformly adhered to. There is also a direct bilirubin which is reported by some laboratories at the end of 15 minutes reaction time before the addition of alcohol.

4-3. In a procedure outlined in AFM 160–49, *Laboratory Procedures in Clinical Chemistry*, the reaction mixture is diluted with an equal volume of methanol. Since the standard is used to calculate concentration of the total, it follows that division by 2 is necessary to calculate the concentration of the direct which is not diluted. In other words, the reaction mixture in the direct tube is twice as concentrated as the tube containing standard. Diluting the alcohol with an equal volume of reaction mixture prevents the precipitation of protein. If protein is precipitated, the mixture clouds and bilirubin is lost because bilirubin attaches itself to protein. It is not generally recommended in routine laboratory work to use serum as dilute as that in the Malloy and Evelyn procedures.

4-4. There is a limitation on the extent to which serum can be diluted with the intention of applying a dilution factor with no corresponding reduction in volume of the other reagents.

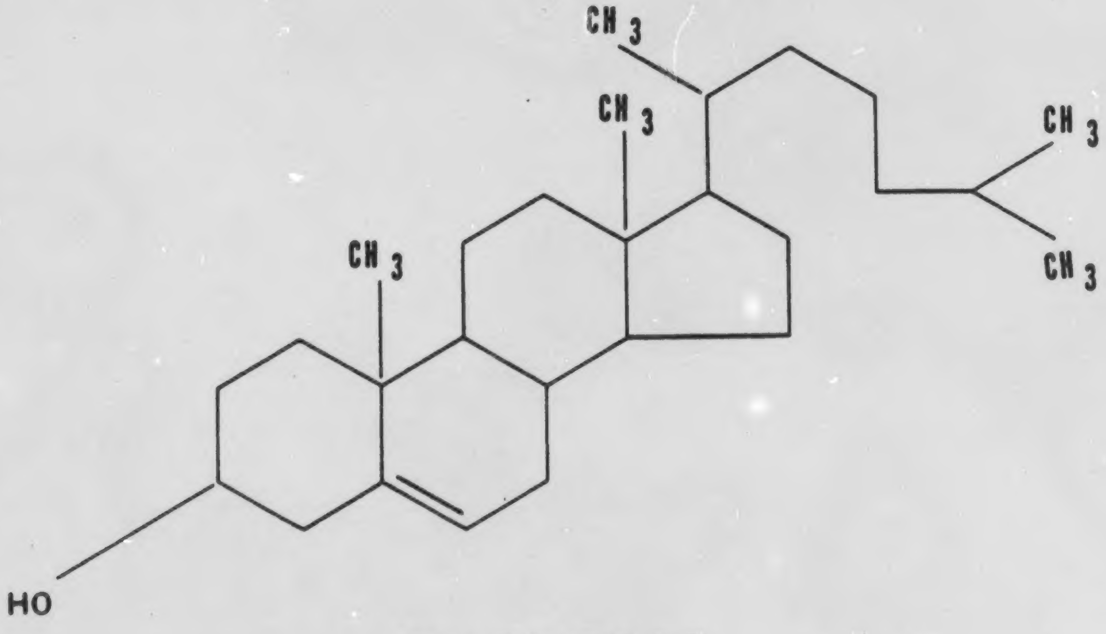


Figure 6. Cholesterol ($C_{27}H_{46}O$).

Every laboratory should establish a valid and reproducible microbilirubin procedure because of the requirement in jaundice of the newborn. Whether it is a diazo method appears to be a matter of choice. If a procedure is scaled down, reproducibility becomes a problem, particularly with the direct bilirubin. An acceptable technique involves the acetone extraction of the unconjugated form of bilirubin from plasma. The resultant extract is spectrophotometrically compared with a standard which meets the requirements set forth in paragraph 4-10. This procedure is discussed in the following paragraphs.

4-5. Six to eight heparinized microhematocrit tubes of whole blood are collected by heel puncture. One end of the tube is sealed and centrifuged for 5 minutes in the microhematocrit centrifuge. After centrifugation, the plasma fraction is expelled into a 10- x 75-mm test tube with a small vaccine bulb. The plasma content of six to eight tubes should be sufficient to provide about 0.25 ml of plasma.

4-6. Add 2.5 ml of freshly prepared 80 percent acetone to each of two 10- x 75-mm test tubes. The tubes are labeled "patient" and "standard." (Eighty percent acetone should be made fresh by diluting 8.0 ml of acetone with 10.0 ml of distilled water.)

4-7. One hundred lambdas of the patient's plasma and 100 lambdas of bilirubin standard are rinsed into the acetone of the respective tubes, using 100 lambdas "to contain" pipettes.

4-8. The tubes are stoppered and mixed well for 3 minutes. Both tubes are centrifuged at 2000 rpm for 5 minutes.

4-9. Transfer the resultant supernatant liquid to appropriately labeled 10- x 75-mm Coleman microcuvettes. Immediately read the optical densities of the tubes at 460 mμ, using 80 percent acetone as the reagent blank.

4-10. Selection of a suitable standard is very important, but unfortunately there is a lack of agreement in our Air Force laboratories as to what constitutes a suitable bilirubin standard. The bilirubin standard recommended by the College of American Pathologists sets forth the following requirements:

a. Bilirubin powder should be accurately weighed and dissolved in *M*/10 sodium carbonate so the sodium carbonate constitutes 2 percent of the final volume of standard.

b. The stock standard is diluted immediately with a serum diluent. Serum diluent is prepared from pooled serum of absorbence less than 0.100 at 414 mμ and normal saline.

c. Factors of time, temperature, and light must be controlled since bilirubin color fades significantly in the light at room temperature. The prepared standard or reconstituted lyphilized standard is stable for 5 days in the dark at 5° C. Keep in mind that bilirubin is not stable in the light at room temperature. Further, a standard must contain an exact weight of bilirubin in a suitable diluent. In the case of bilirubin, the diluent affects intensity of color development. You are undoubtedly aware of this, because some procedures call for an enhancement of color by the addition of various substances.

4-11. **Cholesterol Procedures.** We will now consider the common test procedures for the determination of cholesterol. An attempt is made in the laboratory to identify the same basic fractions of cholesterol which were discussed earlier. Thus, we have developed tests that are more or less specific for free cholesterol and cholesterol esters.

4-12. *Total cholesterol.* Cholesterol and various cholesterol derivatives which are found in serum will react with strongly acidic substances to produce a color. The most popular nonautomated procedure that is performed in U.S. Air Force medical laboratories involves the Lieberman-Burchard reaction. The color development mixture (CDM) in this reaction is composed of acetic anhydride, glacial acetic acid, and sulfuric acid. The solution is not stable for more than 24 hours after sulfuric acid is added to the acid-anhydride. The color developed between cholesterol and CDM in the presence of a small amount of water is blue-green. Stability of this color depends upon the quality of color development mixture. An explosive situation in this regard is a direct mixture of water with acetic anhydride. This may occur inadvertently through the use of wet glassware. Unintentional or not, *a violent reaction* will result. Splashes on the skin or in the eyes should be rinsed *immediately* with copious amounts of water.

4-13. One way to perform a total cholesterol determination is to react the cholesterol reagent directly with serum without prior extraction of the cholesterol with organic solvents. However, the direct use of serum does not reflect carefully controlled test conditions and cannot be recommended. This has been shown to yield results that are higher than those obtained with extraction methods, particularly if bilirubin is present. Extraction of cholesterol with petroleum ether reduces the amount of icteric substances present, though it does not completely eliminate them. A variety of methods for preparing compensating blanks can be found in current literature. Experiments demonstrate that 10 mg-% of bilirubin can elevate a cholesterol result by as much as 200 mg-%. This means an increase of 100 percent over the normal values generally accepted for cholesterol. This is a significant error, which has until recently been discounted in many technical publications. The interference of bilirubin and complexities of the color reaction are responsible for variation in cholesterol results, even among laboratories staffed by qualified analysts.

4-14. Some of the variables, in addition to the presence of bilirubin, include composition of the CDM, time of reaction, temperature, effects of light, and use of lipemic serum. The effects of time and temperature can be partially compensated by selecting a wavelength that provides the most valid measurement. This explains why a wavelength of 540 to 550 is selected, although the color peaks occur at 530 and 630 mμ. The presence of organic solvents, principally chloroform, enhances color sufficiently to yield results that are 10 to 20 percent higher than if chloroform is not present.

4-15. In essence, the most accurate methods for determining cholesterol involve extraction techniques, but even these involve a number of variables that must be considered. Fortunately, the normal values for a serum cholesterol represent a comfortable range within which some variation is expected by the clinician. The most recent figures list 150 to 310 mg-% as normal for total cholesterol, with significant variations in respect to age and sex, which is somewhat higher than values found in AFM 160–49 prior to the 1967 revision. The Schoenheimer-Sperry method, which uses the Lieberman-Burchard reaction, is now considered the reference method for cholesterol values.

4-16. The automation of a colorimetric procedure for cholesterol is based on a method introduced by Zlatkis, Zak, and Boyle in 1953. Much more sensitive than the Lieberman-Burchard reaction, this method results in a yellow color as cholesterol reacts with a mixture of concentrated sulfuric acid and ferric chloride ($FeCl_3$). A 1:10 isopropanol extract of serum is prepared and an aliquot of the extract is diluted with a segmented stream of premixed color reagent. Color is developed in a 95° C. heating bath and measured at 520 mμ in a flow cell. Pulsing is effectively eliminated in the tubing by pulse chambers which are part of the Autoanalyzer system.

4-17. Other methods for the assay of cholesterol which do not use the Lieberman-Burchard reaction are not yet widely accepted but may, someday, become reference methods. The method of Tschugaeff, for example, involves the development of a red color when cholesterol is reacted with acetyl chloride, zinc chloride, and glacial acetic acid. The method of Pearson, described in Chapter 10 of AFM 160–49, measures the color produced by the reaction between cholesterol and cholesterol acetate with p-toluene sulfonic acid in acetic anhydride. The color is proportional to the concentration of total cholesterol. The reaction is for total cholesterol only and cannot be used if digitonin is present or if the serum is hemolyzed. There is some difference of opinion among authorities as to how the Pearson method compares with the standard Schoenheimer-Sperry method. While useful in routine screening of clinical specimens, it should be used with a knowledge of the sources of error previously mentioned in all methods for the direct estimation of cholesterol. NOTE: It is also advisable to substitute 12 percent sulfosalicylic acid for p-toluene sulfonic acid reagent, as suggested by Rappaport and Eichhorn in 1960. This is strongly recommended because of reported *explosions* which have resulted from the use of p-toluene sulfonic acid reagent. It is considered desirable in many cholesterol methods to saponify the cholesterol before reacting it to form a color. In chemistry, *saponification* is the process by which fats are converted into soap and glycerol upon the addition of an alkali. This is usually accomplished with the addition of heat. In the case of cholesterol, saponification has the advantage of yielding one type of cholesterol which produces one type of color. Extraction problems are minimized because it is easier and more reliable to extract saponified cholesterol. Subsequent precipitation of the free cholesterol with digitonin may be retarded. In general, the advantages of saponification have identified it as a more sophisticated approach to the determination of cholesterol.

4-18. *Procedures for cholesterol esters.* Cholesterol esters do not precipitate with digitonides because the hydroxyl group of the ester is not free. Precipitation may be facilitated if aluminum ions are added, usually as $Al(OH)_3$. The Lieberman-Burchard reaction may then be carried out on the washed precipitate which represents the free cholesterol. Esters are calculated as total minus free. You may find it easy to forget that you must subtract free cholesterol from total cholesterol to report the ester fraction.

4-19. **Liver Function Screening Tests.** A few relatively simple tests are frequently used to aid the physician in evaluating the general condition of the liver. Some widely used tests for this purpose are thymol turbidity, cephalin-cholesterol flocculation and the bromsulphalein retention test (BSP).

4-20. *Thymol turbidity test.* Turbidity is caused in a buffered thymol solution when a serum that contains an abnormal globulin is added to the solution. The degree of turbidity is measured photometrically with the Coleman Jr. spectrophotometer at 650 mμ, and the degree of turbidity reported in Shank-Hoagland units. The standard consists of a barium sulfate suspension which is prepared from 0.0962*M* barium chloride and dilute sulfuric acid. The buffer is a thymol-barbital buffer which should have a pH of 7.55. If a pH of 7.8 is used the results will be 30 percent lower. A Shank-Hoagland unit, as described in AFM 160–49, is equivalent to one Maclagan unit. However, it should be pointed out that units are reported by some medical facilities in which one Maclagan unit equals two Shank-Hoagland units because they may use a standard that is 0.0962*N* instead of 0.0962*M*. Thus, clinicians may be familiar with a Shank-Hoagland unit as representing two rather than one Maclagan unit. It should be noted that values listed for many commercial controls are in Shank-Hoagland units, which are valued at twice those given in AFM 160–49.

4-21. *Cephalin-cholesterol flocculation.* Normal serum will not flocculate an emulsion of cephalin, cholesterol, and lipid which is prepared according to the method of Hanger. In liver damage there is an alteration in the protein fraction of serum which causes precipitation that can be quantitatively estimated when serum and antigen are mixed. There is no high degree of standardization among lots of antigen and no other method of standardization is available. However, the test is reproducible for any lot of antigen. The exact nature of protein changes which are responsible for flocculation are not yet agreed upon. It is most likely due to an increase in the gamma globulin fraction and a decrease in the albumin fraction. High serum lipids do not interfere with the cephalin-flocculation test as they do with the thymol turbidity test. The cephalin-cholesterol emulsion is commonly referred to as cephalin-flocculation antigen. The term "antigen" is not completely accurate in describing the cephalin-flocculation reagent because the reaction does not involve antibodies. We usually define an antigen as a substance which causes the formation of antibodies, and this has no application to the emulsion described. The test is usually read as 1+, 2+, 3+, or 4+ in 24 and 48 hours. According to some investigators, an abnormal finding would appear within 24 hours and there would, therefore, seem to be little justification for submitting a 48-hour reading. In fact, a reading at 37° C. at the end of 3 hours has been reported to correlate within acceptable limits with the 24-hour reading.

4-22. Stability of the cephalin-cholesterol emulsion depends to a great extent on the way in which it is prepared. Since the working emulsion is not usually sterile, it should be prepared fresh, as it deteriorates. According to AFM 160–49, the working emulsion should be prepared fresh each day. This is an excellent guideline to insure results of the highest quality. Other sources are more optimistic, but all agree it should not be kept for more than a few weeks and that it should be refrigerated and checked for bacterial and mycotic contamination. All of the well-known sources of error for this test, such as dirty glassware, emulsion preparation, etc., must be recognized for this to be a valid test.

4-23. *BSP.* The BSP test is one which measures the *retention* of bromsulphalein dye by the liver following intravenous injection. The patient is usually in the fasting state when BSP dye is injected. Injection is accomplished by a physician and is not usually undertaken by support personnel or laboratory technicians, because very serious reactions to BSP dye, including death, have been reported. It is customary for laboratory personnel to assist the physician by preparing the syringe and dye. The syringe used to inject the dye must be sterile. The needle used to fill the syringe should be discarded and replaced with a new one prior to injecting 5 mg of dye per kilogram of body weight. The conversion to milliliters of dye can be readily obtained from a chart supplied by pharmaceutical firms who provide the dye or a simple calculation can be used. The patient's weight in pounds divided by 22 is equivalent to the milliliters of 5 percent dye to inject. Blood is withdrawn from the patient at a particular interval thereafter (usually 45 minutes) and serum is used for the test. Whether the vein into which the dye was injected is used as the site for withdrawing blood, or whether the opposite arm is used is of relatively minor importance. The procedure then allows for color development with an alkaline solution and calculation of percent retention with a standard or from a prepared curve. The test is based on the assumption that most BSP dye in the body is removed by the liver and thus serves as an index of liver excretory function. Any blockage of bile flow will impair the ability of the liver to excrete BSP dye. Hence, an increased serum bilirubin, if due to obstruction, will be accompanied by an abnormal BSP test. Photometric interference by icteric serum does not occur significantly if the bilirubin concentration is less than 20 mg-%.

Proteins

PROTEIN CHEMISTRY is a vast and complex study. The relevance of proteins to body functions is so extensive there appears to be no limit which could be clearly set for the clinical chemist in the area of protein chemistry. All body cells, as well as the intercellular material, contain proteins. Hemoglobin, enzymes, and most hormones are protein in nature, as are the immunological mechanisms of the body. This chapter is limited to a few protein determinations that are routinely performed in the clinical laboratory. The changes which proteins undergo in various clinical conditions are far more extensive than can be resolved by a few relatively simple laboratory tests.

2. Because of the complexity of protein changes associated with various diseases, a simple elevation or decrease in total protein or a common fraction is not necessarily of definitive diagnostic value. It does contribute to the clinical information on hand if the physician is aware of changes which occur in the total protein, and albumin and globulin fractions. It might be generally stated that total serum protein usually decreases in abnormal conditions as a result of a decreased albumin fraction. Exceptions to this do occur and changes of other protein fractions, such as globulin components, are measured in most laboratories. This chapter is intended to give you background information which will be used in later chapters of this course and in other CDCs for which this course is a prerequisite. More detailed protein measurements will be discussed in a later chapter in connection with the technique used. Enzymes are also discussed separately.

5. Chemistry and Physiology of Proteins

5-1. Much has been written in recent years about the chemistry and physiology of proteins. Proteins constitute a large portion of blood, muscle, and other tissue. All enzymes and most of the hormones are proteins. In this section we will briefly describe the chemical nature of proteins, followed by a discussion of the application of proteins to physiology and the practice of medicine.

5-2. **Nature of Proteins.** Proteins are large molecules composed of chains of *alpha amino acids*. The general formula for an alpha amino acid is R-CH(NH_2)-COOH. In solutions at the proper pH, amino acids exist in an ionized form. That is, the carboxyl group (COOH) loses a proton to water so that a COO^- group is formed, and the amino group (NH_2) gains a proton. The amino group of an amino acid can react with the acid group (COO^-) of another amino acid to form a peptide linkage. Amino acids combine with each other in long chains, and each may be of the same structure, or different. Chains of amino acids are called polypeptides, the protein molecule being an elaborate polypeptide, as illustrated in figure 7.

5-3. The sequence and characteristics of the amino acids determine the nature of the proteins which they form. Keeping in mind that a protein molecule is three-dimensional, you should realize there is an almost infinite number of ways in which the molecule may be geometrically arranged. The exact arrangement is a significant factor affecting the nature of proteins. Scientists are able to study the dimensional structure of a protein molecule by various techniques using X-ray beams. As an X-ray beam is sent through a crystal from various directions, the pattern produced on a photographic plate will indicate the

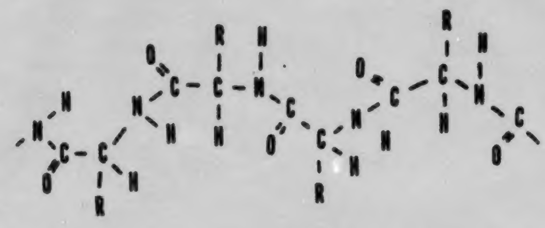


Figure 7. Portion of repeating chain of amino acids in polypeptide linkage.

configuration of the molecules. From this information investigators are able to construct three-dimensional models of a protein molecule. The chains of molecules may be twisted about each other to form a helix pattern, which is characteristic of certain proteins, such as a collagen molecule diagrammed in figure 8. There are, of course, many other three-dimensional configurations. At the present time more than 20 different amino acids have been isolated from proteins upon hydrolysis. The possible ways in which over 20 amino acids can combine suggest a wide variety of proteins, and indeed there are. A change in the sequence or position of one amino acid alters the protein.

5-4. There are various ways of classifying proteins, though no system has been devised which is beyond criticism. Proteins that contain amino acids, or their derivatives, and no other substances are frequently classified as simple proteins. Those which contain a nonprotein substance, called a prosthetic group, are referred to as conjugated proteins. Albumin and globulin are simple proteins, whereas chromoproteins (hemoglobin), mucoprotein (mucin), phosphoproteins, and lipoproteins are examples of conjugated proteins. Because of the large size of a protein molecule, proteins behave as *colloids* in water. Colloids are particles held in aqueous suspension due to a marked attraction between the water molecules and protein molecules. Suspended molecules of protein are also called hydrophilic or lyophilic colloids. Proteins have the interesting characteristic of changing charge—being electrically positive, negative, or neutral as conditions vary. Substances which act as both acids and bases because of a duality of electrical charge are described as *amphoteric* or *amphoterous*. The protein molecule is amphoterous because it bears a charge dependent upon the pH of the solution in which the protein occurs. The electrical behavior of proteins will be further described in another chapter in the context of protein electrophoresis.

5-5. Properties of proteins were described in some detail as early as 1807 by the Russian investigator, Reuss. Then, in 1861 the migration of proteins in an electrical field was related to the pH of the solution. It was discovered later that electrical properties of proteins are conferred upon them by the alkaline or acid nature of the medium. The pH at which proteins are electrically neutral is called the *isoelectric point*. Obviously, the isoelectric point will be different for different proteins because all protein fractions do not have the same charge characteristics. You should recall that proteins remain in solution as a result of the attraction of protein molecules for molecules of the solvent. This force is opposed only by the attraction of protein molecules for each other. The greater the charge on the molecules, the less significant will be the force opposing suspension. At the isoelectric point, the net charge of the proteins will be zero and the protein molecules will, therefore, cease repelling each other. If the protein is in a salt solution of optimal concentration at its isoelectric point, the salt molecules will compete for the water and become hydrated. Conversely, the protein molecules will become dehydrated and precipitate. This is the chemical basis of "salting out" techniques which will be discussed later in this chapter. It is a useful principle that can be applied to separate, differentially, one protein from another in colloidal suspension.

5-6. **Physiology of Proteins.** Proteins that are taken into the body in the form of various foods begin to break down by the action of pepsin in the stomach. Breakdown of the large protein molecules in the stomach results in proteoses and peptones, as well as some amino acids, which are the basic units. Further breakdown of proteins, proteoses, and peptones occurs in the intestine under the action of the enzymes, trypsin, and chymotrypsin from the pancreas. Polypeptides which are not hydrolyzed to amino acids by the activity of other enzymes are broken down to amino acids by peptidase, including carboxypeptidase, also from the pancreas.

5-7. Proteins are absorbed into the blood in the form of amino acids and a few relatively short

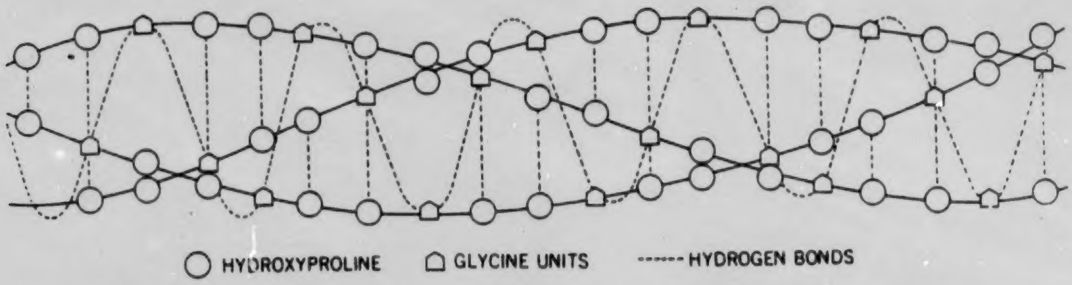


Figure 8. Diagram showing helix structure of a protein molecule.

peptide chains. The absorption of large protein molecules may result in the production of antibodies as a systemic response. Amino acids absorbed into the blood are immediately taken up by the tissues with no appreciable increase in the plasma amino acid level. The amino acids are then used for protein synthesis, stored in a phosphorylated form, or deaminated in the liver. Some may be used for special purposes.

5-8. Proteins of the body are constantly being broken down and re-formed. The processes by which proteins are synthesized in the body are still undergoing investigation. It is sufficient to state here that protein metabolism is a dynamic process which is constantly taking place, and involves the transfer of energy by means of the high energy phosphate bond mentioned in an earlier chapter. Proteins may be used for energy if the intake of carbohydrates and fat is inadequate, or if a metabolic dysfunction occurs. In starvation there is a wasting away of muscle tissue as proteins of the muscle are catabolized to produce energy. In addition to the necessary amino acids for protein synthesis, dietary proteins furnish the amino acids necessary for the synthesis of various body compounds other than body proteins. For example, the amino acid, glycine, converts benzoic acid to hippuric acid, the latter being excreted by the kidney. As will be noted in a discussion of enzymes, amino groups are frequently transferred from one amino acid to another by the process of *transamination*. There are many possible ways of converting various amino acids as they are required by the body. Ammonia groups which are not transferred to an amino acid are converted into urea. Urea is excreted via the kidneys.

5-9. Amino acids, as such, are excreted only in trace amounts in the urine. Excretion of amino acids to any significant extent suggests a pathological condition, such as abnormal protein metabolism or tubular resorption problems. Many of the metabolic disorders are hereditary. One such common disorder familiar to laboratory technicians is phenylketonuria (PKU) which results from an accumulation of phenylalanine. If undetected very early in life, this metabolic disorder results in irreversible brain damage. The laboratory test for PKU is included in the volume covering urinalysis, but is worth mentioning here as a disorder of protein metabolism. Albinism is another disorder of protein metabolism; and is, in some ways, analagous to diabetes, which is a disorder of carbohydrate metabolism. A most interesting genetic trait associated with proteins is involved with sickle-cell anemia. In this inherited disease the substitution of one amino acid (valine) for another (glutamic acid), among over 500 amino acids in the molecule, produces the abnormal "S" hemoglobin.

5-10. Another relevant syndrome familiar to the laboratory worker is a tumorous condition of the intestinal tract characterized by the presence of 5-hydroxyindolacetic acid (5-HIAA) in the urine. The compound, 5-HIAA, is derived from serotonin which is an amino acid (5-hydroxytryptamine). Thus, we have another familiar example of faulty protein metabolism. Through the process of synthesis, the body is able to produce all but 8 of the 20 amino acids. These eight, sometimes called the essential amino acids, are: threonine, valine, leucine, isoleucine, methionine, lysine, tryptophane, and phenylalanine. If the diet is deficient in any of the eight amino acids listed, the individual will develop various symptoms of protein deficiency.

5-11. Biochemists have studied the structure of proteins much more extensively than the nature of molecules which participate in the body synthesis of proteins. One group of control molecules is the ribonucleic acid (RNA) compounds. RNA is a complicated chemical structure, formed from pentoses, phosphoric acid, and nitrogen bases. RNA plays a crucial role in each step of the process of protein synthesis within the cells. The key to understanding how a nucleic acid molecule is able to control synthesis is the structure of the nucleic acid molecule itself. One of the first molecules studied in detail was one of the short RNA molecules which transports the amino acid, alanine. The nucleic acid components, known as nucleotides, were described in detail for this particular molecule and reported for the first time in 1965. When we consider that this is one of the protein synthesis control molecules structurally elaborated, we realize that the biochemistry of proteins requires many years of further study. The complex functions of another nucleic acid group called deoxyribonucleic acid (DNA) also continues to be studied extensively, particularly with respect to hereditary control. Other factors that control protein synthesis include the many enzymes which, as stated previously, are themselves proteins.

6. Laboratory Investigation of Proteins

6-1. Methods of protein analysis outlined in AFM 160-49, *Laboratory Procedures in Clinical Chemistry*, are discussed in the following paragraphs. Information concerning protein levels are of diagnostic value to the physician, although protein functions are so numerous and complex that other laboratory tests, as well as the usual clinical evaluations, may be necessary to establish a definitive diagnosis. Besides data concerning total protein and the A/G ratio, the physician may be concerned with other protein elements, such as fibrinogen or hemoglobin. A complete analysis of fractions, including albumin and globulin, is usually done by electrophoresis which will be discussed in a later chapter.

6-2. **Biuret Method for Total Protein.** The method of Gornall, Bardawill, and David presented in AFM 160–49 for the determination of total serum protein has proved very satisfactory. The protein in serum is reacted with an alkaline copper sulfate, in the presence of ethylenediaminetetra-acetic acid, to form a blue-violet complex (biuret reaction). Unlike some of the earlier biuret mixtures, that which is used in the Gornall method rarely produces a turbid solution. Some of this turbidity is caused by precipitation of calcium and magnesium hydroxide in alkaline solution. This is prevented when Na_2EDTA is included in the biuret reagent. If turbidity does develop, it is probably from lipids, which may be extracted with ether. Several different modifications of the original biuret method are used today. The formula used by most U.S. Air Force facilities is as follows:

Copper sulfate ($CuSO_4 \cdot 5H_2O$)	1.5 g
Disodium ethylenediaminetetra-acetate (Na_2EDTA)	6.0 g
Potassium iodide (KI)	1.0 g
Sodium hydroxide, 2 • 5N	300.0 ml
Distilled water – qs to	1000.0 ml

6-3. A number of commercial preparations are available which have the name "biuret reagent." They all contain copper sulfate, but some may contain other stabilizing agents, such as citrate or NaOH instead of KOH. Increased stability has been reported by substituting Na_2CO_3 for the NaOH (or KOH) and then adding the hydroxide separately before using the working solution. There are two distinct problems that must be overcome in stabilizing biuret reagent. The first is that $Cu(OH)_2$ tends to precipitate from solution. The second difficulty is that a highly alkaline solution of copper sulfate can be easily reduced if contaminated. In any event, if a stable biuret reagent is used, the biuret method generally produces satisfactory results. The biuret method may be applied to protein analysis in cerbrospinal fluid, but is less sensitive at lower protein concentrations. Urine proteins are not usually assayed by this method unless the protein is first separated from interfering substance by precipitation. Bile pigments (bilirubin) are reported to interfere with this reaction only if their concentration is greater than 30 mg-%. Hemoglobin will react with biuret reagent if the specimen is hemolyzed.

6-4. **Precipitation Methods.** The precipitation of proteins by acids to produce a turbid solution is a useful concept with urine and cerebrospinal fluid. This is not the technique used for serum which requires a more precise method suitable for the relatively high concentration of protein in serum or plasma. In the classical method of Kingsbury and Clark for urine proteins, the protein was precipitated with sulfosalicylic acid after clearing the urine of insoluble phosphates with acetic acid. More recently, trichloroacetic acid (TCA) is substituted for sulfosalicylic acid. This is because sulfosalicylic acid tends to give strikingly different turbidities with albumin and globulin. Specifically, above 23° C., albumin gives much more turbidity than an equivalent concentration of globulin. Below this temperature, albumin yields less turbidity than globulin. Total protein results, therefore, become a function of the A/G ratio of the urine rather than the absolute amounts of albumin and globulin contained in the urine. This method was described in a procedure disseminated by the USAF Epidemiology Laboratory in 1965. The turbidity produced is an indication of the amount of protein present in the specimen. Unfortunately, the protein particles flocculate rather rapidly after the precipitate is formed. To be measured reliably in the spectrophotometer, the precipitate should be fine, not a heavy flocculation. This problem may be overcome by breaking up the floc mechanically by shaking or by the addition of gum ghatti. If gum ghatti is used, a corresponding blank must also be used. A calibration curve is usually prepared using a suitable protein standard such as the standard available through regular supply channels. By measuring the 24-hour volume of urine, you can easily relate the concentration of an aliquot of the unknown to protein excretion per 24-hour period with the following formula.

$$\text{mg protein per 24 hr} = \text{mg-\% protein} \times \frac{\text{volume of 24-hr specimen}}{100}$$

6-5. You should be assured that a specimen submitted for a 24-hour analysis of any kind is in fact a 24-hour specimen. As a guide, you may keep in mind that the normal adult 24-hour urine volume is 1200 to 1600 ml. It is advisable to report the 24-hour volume together with the test results on the laboratory report form. Refrigeration or addition of toluene is usually adequate to preserve the specimen prior to analysis. The TCA precipitation method may also be applied to spinal fluid protein.

6-6. Many laboratories test cerebrospinal fluid (CSF) by the Pandy qualitative test for globulin on the assumption that any significant increase in globulin can be so detected. Recent studies have shown that solutions of phenol are unreliable and not specific for globulin. Hence, the Pandy globulin test has been abandoned by many progressive laboratories. The normal spinal fluid protein is from 15 to 45 mg–%. You might assume that since qualitative protein "sticks," used in screening urine specimens, are sensitive from about 30 mg–%, they would also be ideal for screening elevated spinal fluid protein. Unfortunately, this is not the case, because spinal fluid proteins are approximately 50 percent globulins to which the strip indicator, tetrabromphenol blue, is much less sensitive. In fact, levels of globulin from 50 to 100 mg–% have not been reliably detected by this test. However, the usefulness of protein test sticks in screening urine specimens is not precluded by this problem. Further, no manufacturer has ever advocated the use of this product for cerebrospinal fluid. A phenol method is currently used which involves a reaction between phenol and a phosphotungstic-phosphomolybdic acid reagent. It is a reasonably sophisticated procedure which is reported to be 100 times more sensitive than the biuret method. Sensitivity is, of course, a consideration with low protein values.

6-7. **Establishing the A/G Ratio.** The best method of determining serum protein fractions is by electrophoresis. Relatively few Air Force laboratories are authorized the expensive equipment necessary for electrophoresis, and it is therefore, desirable that a method be available to at least assay the albumin and globulin fractions. The clinical importance of the A/G ratio is well known, being of particular value in liver disease, congenital anomalies, and malignancies. Some diseases and their usual protein patterns are listed in table 1. The importance of determining more than just total protein should be clear to laboratory personnel, although clinical interpretation is not the concern of the laboratory. You should know the normal serum values which are:

Total protein	6 to 8 g per 100 ml
Albumin	3.6 to 5.6 g per 100 ml
Globulin	1.3 to 3.2 g per 100 ml
A/G ratio	1.5:1 to 2.5:1

6-8. There is considerable confusion in terminology as to exactly what constitutes globulin. As techniques of electrophoretic separation have become refined, more specific fractions are being resolved and arbitrarily identified as albumin components or globulin components, depending upon their rate of migration in an electrical field. It becomes difficult, if not impossible, to transfer electrophoresis terminology to protein fractions separated by less refined techniques. One such technique, called "salting out" is explained in the following paragraph with the understanding that a perfect parallel does not exist between fractions separated by this method and albumin or globulin fractions identified by other techniques. However, the correlation is sufficiently close to permit meaningful interpretation of the results.

6-9. In "salting out," advantage is taken of the fact that certain proteins are not soluble at their isoelectric points. Upon the addition of a neutral salt, such as sodium sulfate (Na_2SO_4) or sodium sulfite (Na_2SO_3), certain proteins will precipitate from solution. As concentration of the salt varies, the fraction which is precipitated from solution will vary. It has been determined that upon addition of 26 percent Na_2SO_4 or a slightly higher concentration of sodium sulfite, a fraction identified as globulin will precipitate from an aqueous solution of serum proteins. Within the past few years the use of Na_2SO_3 has largely replaced Na_2SO_4 because sodium sulfite agrees better with electrophoretic patterns. The use of sodium sulfite has the additional advantage of increased solubility at room temperature. The April 1967 revision of AFM 160–49 suggests using 9.5 ml of 28 percent sodium sulfite and 0.5 ml of serum. This is an error which will be corrected in the next revision, because it results in a concentration of Na_2SO_3 which is less than the optimum 26.9 percent. The optimum concentration of 9.6 ml of Na_2SO_3 to which 0.4 ml of serum is added should be 28 percent. This results in a sulfite concentration of 26.9 percent in the serum-sulfite mixture. The original Howe method used 22.2

TABLE 1

CLINICAL CONDITIONS AND CHARACTERISTIC ALBUMIN-GLOBULIN FRACTIONS OF SERUM

CONDITION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A/G
Multiple myeloma	Increased	Normal	Increased	Decreased
Dehydration Hemoconcentration	Increased	Increased	Increased	Normal
Certain liver diseases	Normal	Decreased	Increased	Decreased
Kidney damage Malnutrition Malabsorption syndromes	Decreased	Decreased	Normal	Decreased

percent sodium sulfate, which resulted in loss of alpha globulin with the albumin. With methods using 26 percent sodium sulfate, the solution becomes saturated at room temperature and must be kept at incubator temperature. The same concentration of sodium sulfite, on the other hand, is not quite saturated at room temperature; and is, therefore, easier to work with. The sulfite is less stable, since it deteriorates as oxygen is absorbed from the air.

6-10. After the globulins have been precipitated with the sulfite mixture, ether is added, together with a few drops of aerosol, to cause the globulin to form a mat, as pictured in figure 9. Albumin remains in solution. The solution containing albumin may be analyzed by the biuret method. The figure calculated as representing the albumin fraction is then subtracted from the total protein to calculate the globulin. Similar salting out techniques are used to identify other specific fractions. For example, an ammonium sulfate reageant can be used to identify the gamma globulin fraction, and 12.5 percent Na_2SO_3 is commonly used to separate fibrinogen, as noted in paragraph 6-12.

6-11. Besides salt precipitation and electrophoresis, other methods are in use for the assay of protein. The classic Kjeldahl method measures protein nitrogen which is essentially an index of the amount of protein present. The Kjeldahl method is frequently referred to in standardizing other methods. An entirely different concept involves the use of dyes to which proteins have specific affinities. One method reported in 1954 used the dye binding capacity of albumin with 2-(4′-hydroxyazobenzene) benzoic acid (HABA). In 1956 an ethylenediaminetetracetate (EDTA) buffer was proposed to stabilize the dye reagent. This modification was applied to the Autoanalyzer in 1966 as an automated method of albumin analysis.

6-12. **Fibrinogen.** Normal human plasma contains 0.15 to 0.30 g–% fibrinogen, which is the important protein element that makes up a fibrin clot. The estimation of fibrinogen is a routine test performed to investigate clotting disorders. Its value is not limited to rare blood dyscrasias, because fibrinogen deficiencies frequently occur in conditions such as complications of pregnancy involving severe bleeding. Various techniques are available for the assay or estimation of fibrinogen, some of which are available as "kits" from commercial sources. In one method, 1 ml of freshly drawn blood is mixed with 0.1 ml topical thrombin (1,000 NIH units per ml). The degree of clot formation is an index of the fibrinogen content. One entire category of fibrinogen tests depends upon the recovery of fibrin, the amount of which is dependent upon the amount of both fibrinogen and prothrombin. The use of a neutral salt (12.5 percent Na_2SO_3) is another means of assaying plasma for fibrinogen similar to the differential precipitation of globulin. Quantitative methods may be applied to the precipitated fibrinogen, one of which is assay of the protein nitrogen content.

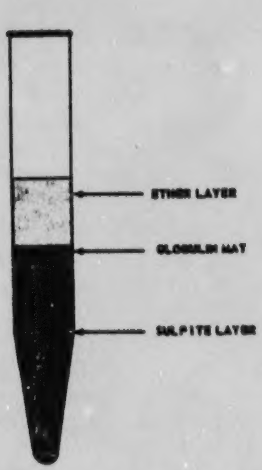


Figure 9. Diagram of tube in which globulins have been "salted out" from a serum protein solution with 26.9 percent Na_2SO_3.

6-13. **Hemoglobin.** Most of the protein in blood is in the form of hemoglobin. The molecular weight of hemoglobin is 64,500 amu. It is composed of about 10,000 atoms. Four of these atoms are iron, each capable of combining with two atoms of oxygen. Each atom of iron in the hemoglobin molecule is surrounded by a group of atoms which form the pigment called *heme*. The heme groups, in turn, are surrounded by a chain of amino acids. There are four amino acid chains which constitute the protein or globin part of the molecule. Globin is a histone which yields histine upon hydrolysis. In all four chains there is a total of 574 amino acids in the hemoglobin molecule. It should be kept in mind that hemoglobin not only carries oxygen but also transports carbon dioxide to the lungs. Carbon dioxide which is carried by the cell is bound to the globin portion of the molecule. The subject of hemoglobin is covered in the course material on hematology, and is merely mentioned here as an important blood protein.

CHAPTER 4

Carbohydrates

IF ONE HAD TO choose the most indispensable chemical determination performed in the clinical laboratory, the choice would probably be the test for blood glucose. Laboratories that perform relatively few chemical determinations will sooner or later perform a test for glucose. Larger laboratories are likely to perform not only many glucose determinations but other carbohydrate studies chemically related to glucose. This might include lactose, xylose, or protein-bound carbohydrates, to mention just a few tests that may be done in the clinical laboratory.

2. In addition to prominence and wide application, carbohydrate analyses rank among the first body chemistries performed in the laboratory. Consequently, the field of carbohydrate chemistry is reasonably well developed and involves many different approaches and contributions over the years. A comprehensive review of carbohydrate chemistry would require many volumes, even if limited to clinical application. This chapter will be limited to a brief discussion of carbohydrate chemistry and the most commonly performed tests for glucose. Refer to a current index of technical and medical literature for an insight into the wide application of other carbohydrate tests which are, in many instances, of great clinical value. While many of these can be performed in the average laboratory, they are not included in the following limited discussion.

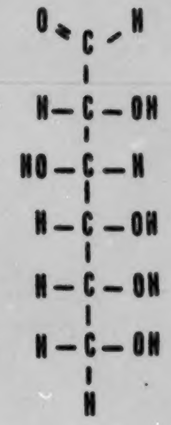
7. Chemistry and Physiology of Carbohydrates

7-1. Carbohydrates, or saccharides as they are commonly known, are of wide interest in clinical medicine. Glucose, a monosaccharide with the formula $C_6H_{12}O_6$, is of particular interest to the clinical laboratory. It is in the form of monosaccharides that carbohydrates are absorbed into the blood. Various deficits in absorption, metabolism, or in the regulatory mechanisms (such as endocrine disorders) are diagnosed and treated on the basis of the blood glucose level. For example, a deficiency of insulin, which is produced by the pancreas, causes diabetes mellitus. Carbohydrates occur in various forms, known as isomers, which will be explained at some length in this section, because isomerism is a fundamental aspect of carbohydrate chemistry. Information presented in this chapter will also be of benefit to you in your study of bacteriology by enabling you to better understand carbohydrate fermentations.

7-2. **Classification of Carbohydrates.** Glucose belongs to the chemical family of carbon containing compounds known as *saccharides* or *saccharoses*, which are represented by the formula $C_xH_{2y}O_y$. They do not contain atoms of other elements. From this formula it is apparent that saccharides contain hydrogen and oxygen in the same 2:1 ratio as they occur in water. Hence, the older term "carbohydrate", rather than "saccharide," is often used. The saccharides are classified according to structure. *Monosaccharides* have the simplest structure with the formula $C_xH_{2y}O_y$. They do not hydrolyze into other sugars, but remain unchanged. Glucose is an example of a monosaccharide with the formula $C_6H_{12}O_6$. *Disaccharides*, with the formula $C_n(H_2O)_{n-1}$, yield two simple sugars of the same or different type upon hydrolysis. Some examples of the disaccharide group are sucrose, lactose, and maltose, all with the formula $C_{12}H_{22}O_{11}$, as represented graphically in figure 10. Sugars with more than two saccharide groups are referred to as polysaccharides, some of which can be designated as $(C_6H_{10}O_5)_n$. If n is less than 10, the term "oligosaccharide" is sometimes used. Starch, dextrin, cellulose, and glycogen are all examples of polysaccharides. They are less like common monosaccharides and disaccharides, in that high molecular weight saccharides are not sweet, are less soluble, and form colloidal solutions which cannot be dialized.

7-3. Within each of the three major categories (i.e., mono-, di-, and polysaccharides), carbohydrates are classified according to the number of carbon atoms each component sugar possesses. As indicated by the formula $C_6H_{12}O_6$, the monosaccharides of greatest biological interest have 6

D-GLUCOSE: A MONOSACCHARIDE



SUCROSE: A DISACCHARIDE COMPOSED OF GLUCOSE AND FRUCTOSE

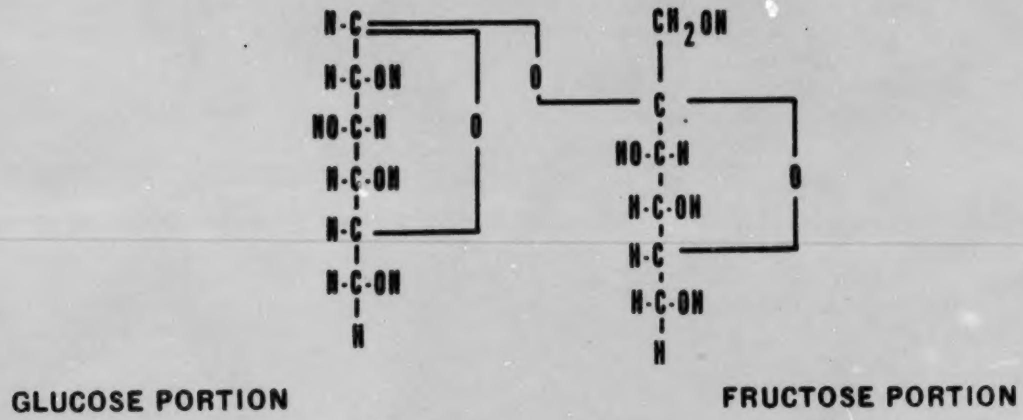


Figure 10. Structural formulas of a monosaccharide, D-(+) glucose, and a disaccharide sucrose.

oxygen atoms. Accordingly, they are classified as hexoses. Glucose, the hexose most common to the clinical chemist, is also called dextrose.[13] But other monosaccharides besides the hexoses are known to the organic chemist and are of less interest to the clinical chemist. For this reason, the general formula for monosaccharides, $C_nH_{2n}O_n$, was given rather than $C_6H_{12}O_6$, which is limited to hexoses. Table 2 illustrates how some carbohydrates are classified.

7-4. **Stereoisomerism.** Carbohydrates occur as isomers. Isomers are forms of a substance which differ in atomic arrangement. The phenomenon of isomer occurrence is termed "isomerism." Stereoisomerism is due to spacial rather than structural arrangement, and occurs in two common forms: geometric and optical. Geometrical isomers are formed because some factor (as a double bond) prevents free rotation of the atoms within the molecule. The isomers formed by geometric isomerism are termed "cis-" and "trans-," and the difference is shown below in the diagrams of two geometric forms of butenedioic acid:

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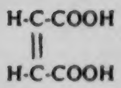
[13] Dextrose should not be confused with *dextrin*, which is a polymer from partially hydrolyzed starch. Starch, cellulose, glycogen, and dextrin are collectively classified as *dextran* and are all polysaccharides.

TABLE 2

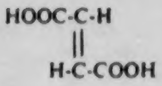
CLASSIFICATION OF CARBOHYDRATES

(Incomplete Listing)

- **Carbohydrates**
  - **Sugars**
    - **Monosaccharides**
      - **Dioses ($C_2H_4O_2$)**
      - **Trioses ($C_3H_6O_3$) -glycerose**
      - **Tetroses ($C_4H_8O_4$) -erythrose**
      - **Pentoses ($C_5H_{10}O_5$) -arabinose**
      - **Hexoses ($C_6H_{12}O_6$) -glucose**
      - **Heptoses, Octoses, etc.**
    - **Disaccharides**
      - **Sucrose, lactose, maltose, cellobiose, etc. ($C_{12}H_{22}O_{11}$)**
      - **Glucoxylose, etc. ($C_{11}H_{20}O_{10}$)**
  - **Nonsugar**
    - **Trisaccharides ($C_{18}H_{32}O_{16}$) -raffinose**
    - **Tetrasaccharides ($C_{24}H_{42}O_{21}$) -stachyose**
    - **Polysaccharides ($(C_6H_{10}O_5)_n$)**
      - **Dextran (dextrins, glycogen, starch, cellulose)**
      - **Galactosans, etc.**



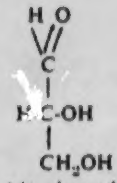
cis-form



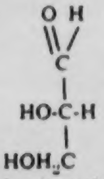
trans-form

The double bond illustrated prevents the atoms from rotating; thus there are two distinctly different geometric possibilities.

7-5. An asymmetric atom (in our study it will always be a carbon atom) is one which has a different atom or radical attached to each of its four valence bonds. Glyceraldehyde, in the formula below, has one asymmetric carbon atom. This carbon has attached to it a hydrogen, a hydroxyl group, an aldehyde group, and an alcohol group. The isomers formed in optical isomerism are mirror images of each other. Looking again at glyceraldehyde, we see that there are two optical isomers, each a mirror image of the other. Such isomers are called enantiomorphs (from the Greek meaning "opposite form").



(d) glyceraldehyde

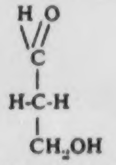


(l) glyceraldehyde

In naming these compounds, the position of the OH group nearest the end opposite the carbonyl group (C=O) determines the *(d)* or *(l)* form, as shown above with glyceraldehyde.

7-6. In early studies it was erroneously believed that the two variations in spacial configuration always correlate with optical rotation. Optical isomers differ in their ability to rotate polarized light, due to asymmetrical or crystalline structure. Polarized light is defined as light which vibrates in only one plane. It is produced as illustrated in figure 11. When polarized light is passed through a solution of an optically active substance, the plane of polarization is diverted either to the right or to the left. Thus, for each carbohydrate there would theoretically exist two optical isomers based on molecular configuration. Compounds which conform to the (*d*) structure when in solution were believed to be those which rotate polarized light to the right, and were called dextrorotatory. Those which, based on their structure, were thought to rotate polarized light to the left were known as levorotatory or the *(l)* form. A mixture of 50 percent each of the (*d*) and (*l*) isomers produces no rotation and is known as a racemic mixture.

7-7. As pointed out, optical properties do not show a clear correlation with spacial configuration. Many optically active compounds do not rotate polarized light as predicted according to structure. Rather, some of the (*d*) forms rotate polarized light to the left. As a result, a second system of nomenclature became necessary to describe the effect on polarized light. Refer again to the structure of (*d*) glyceraldehyde.



(*d*) glyceraldehyde

Under the second system, the compound (*d*) glyceraldehyde becomes *d* (+) glyceraldehyde, because it has the (*d*) structure and rotates polarized light to the right as indicated by a plus sign. If this compound were to rotate the light to the left, it would be called *d* (−) glyceraldehyde.

7-8. Each asymmetric carbon atom can yield two enantiomorphs, and as the number of asymmetric carbons increases, the number of isomers increases. The possible number of enantiomorphs for a compound is 2^x, in which the x stands for the number of asymmetrical carbons. The biological activity of carbohydrates depends upon the symmetrical arrangement of the carbon atoms. For this reason, you should understand the meaning of symmetry and its relationship to isomerism. Optical properties, particularly with reference to polarized light, provide a means of studying and distinguishing between the various isomers.

7-9. **Digestion and Absorption of Carbohydrates.** Polysaccharides and disaccharides are broken down by enzymes into monosaccharides before absorption occurs. Maltase, sucrase, and lactase break down maltose, sucrose, and lactose, respectively, in the intestine. Salivary and pancreatic amylase change starch to maltose. Most of the monosaccharide absorption takes place in the small intestine at a fairly constant rate. The rate of absorption is also characteristic of the particular monosaccharide; and is, therefore, not entirely a matter of simple diffusion, but rather a selective process dependent upon the activity of the intestinal mucosa. The absorbed sugar is picked up by blood capillaries and transported via the portal vein to the liver. Within the liver, the blood carrying the sugar flows through a capillary network and the sugar is taken up by the liver cells.

7-10. **Metabolism of Carbohydrates.** Glucose is used in one of three ways: for energy, for storage after conversion to glycogen, and for conversion to other substances. In addition to glucose from carbohydrates absorbed in the diet, glycogen may be formed from glucose which has been synthesized in the liver from noncarbo-

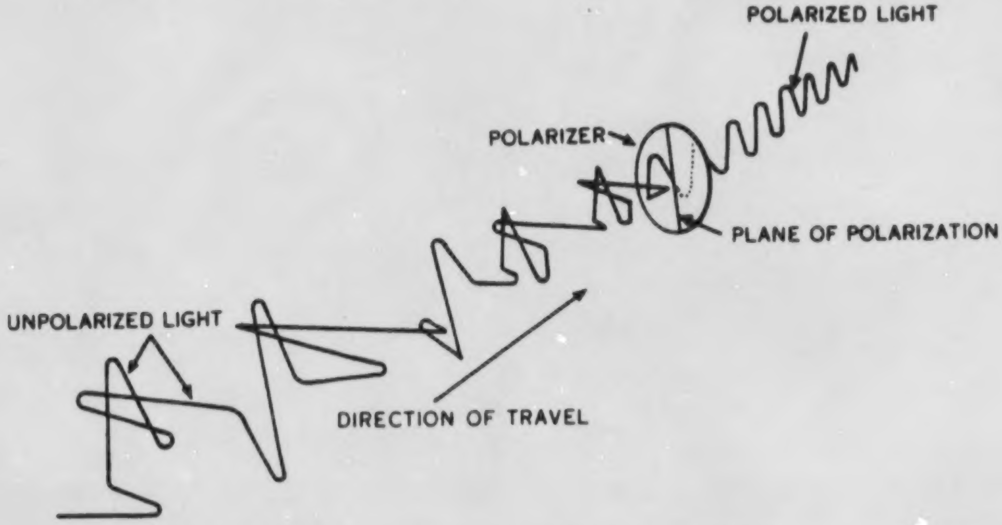


Figure 11. Diagram illustrating principle of polarization.

hydrate sources. The process of glucose formation from noncarbohydrates is called gluconeogenesis. Glucose may be converted to carbohydrates, such as ribose or noncarbohydrate substances, e.g., amino acids. Let's take a closer look at the processes involved in the use of glucose for energy. For convenience, let's describe the breakdown of glucose as aerobic and anaerobic. In aerobic metabolism, glycogen (or glucose) is carried through a series of carbohydrate-phosphate complexes to pyruvic acid, which then oxidizes to carbon dioxide and water.[14] In the anerobic metabolism of glucose (glycolysis), pyruvic acid is reduced to lactic acid. Certain other pathways are available for the breakdown of glucose, including the so-called pentose shunt in which a ribose-5-phosphate or ribulose-5-phosphate compound is formed intermediate to certain other steps and the Krebs cycle. It should be pointed out that metabolism of glucose is not limited to the liver. Muscle plays an active role in carbohydrate metabolism. Both the formation of glycogen (glycogenesis) and the breakdown of glycogen (also called glycolysis) take place in the muscle in a manner similar to reactions which take place in the liver. The obvious purpose for muscle glycolysis is to supply energy to the muscle.

7-11. **Blood Glucose Levels.** The normal fasting glucose level of the blood is 70 to 100 mg–%. This level is often referred to as the true glucose level to distinguish it from figures which include nonglucose reducing substances. The Folin-Wu procedure which produces normal fasting glucose levels of 80 to 120 mg–% includes other reducing substances. Since the blood glucose levels depend upon both the rate at which glucose enters the blood and the rate of glucose removal, levels depend upon various factors. These factors are insulin, epinephrine, pituitary hormones, and thyroxin.

7-12. *Insulin.* This hormone is secreted by the pancreas, and regulates both the rate of glucose output from the liver and the rate of glucose utilization. It also regulates the transfer of glucose across cell membranes. The relationship between diabetes mellitus and the pancreas was accurately described in the late 1800s. However, it was not until 1922 that insulin was discovered, and it is only recently that chemists have been able to synthesize insulin in the laboratory. An excess of insulin, known as *hyperinsulinism,* causes a decrease in blood glucose levels (hypoglycemia). *Diabetes mellitus* is a metabolic disorder resulting from a lesion of the pancreas or overactivity of the pituitary and adrenal glands, which has a direct effect on the pancreas. It is characterized by a lack of insulin, i.e., *hypoinsulinism*. Glucose often appears in the urine, and blood sugar levels are abnormally high (hyperglycemia) in patients with diabetes mellitus.

7-13. *Epinephrine*. Produced by the adrenal gland, epinephrine regulates the rate of glycogen breakdown in the liver. Epinephrine is also called adrenalin. In adrenal cortical insufficiency the blood sugar level is frequently below normal.

7-14. *Pituitary hormones*. Hormones from the pituitary gland, which is attached to the base of the brain, retard the use of glucose in the body; hence, their presence tends to raise blood glucose levels. A head injury or tumor of the pituitary may produce *diabetes insipidus*. In this condition the blood sugar is not necessarily elevated and there is no sugar in the urine, but urine is excreted in copious amounts and has a low specific gravity. The term "diabetes" means an excessive discharge of urine, from the Greek words for "siphon through." The effect of anterior pituitary insufficiency is essentially the same as adrenal insufficiency.

7-15. *Thyroxin*. Regarded as the principal active component of the thyroid hormone, thyroxin increases the breakdown of glycogen in the liver. Hyperthyroidism results in a decreased tolerance for glucose. The process of glucose production and the use of glucose are in dynamic equilibrium to maintain a fairly constant level of glucose in the blood. The regulatory mechanisms are such that a predictable curve can be established to depict the rise and fall in blood sugar levels following the ingestion of glucose. Known as a glucose tolerance curve, a graphic representation of glucose levels at intervals after an intake of glucose or other carbohydrates has great diagnostic significance. In a normal response to glucose intake, the ½-hour level should exceed the fasting level by less than 75 mg–%. Further, the 1-hour level should be less than the ½-hour level, and all urine specimens collected during the test should be negative for glucose. Three different glucose tolerance curves are shown in figure 12.

7-16. Various abnormal glucose tolerance curves and their clinical importance are matters of diagnostic concern more related to the interpretation of laboratory results than to the performance of them. Besides occurring in diabetes mellitus, increased glucose levels may be found in uremia, nephritis, hyperthyroidism, during pregnancy, and in infectious states. As previously mentioned, low blood glucose levels may

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[14] The series of steps involved in the aerobic metabolism of glucose is called the Krebs cycle, tricarboxylic acid (TCA) cycle, or citric acid cycle.

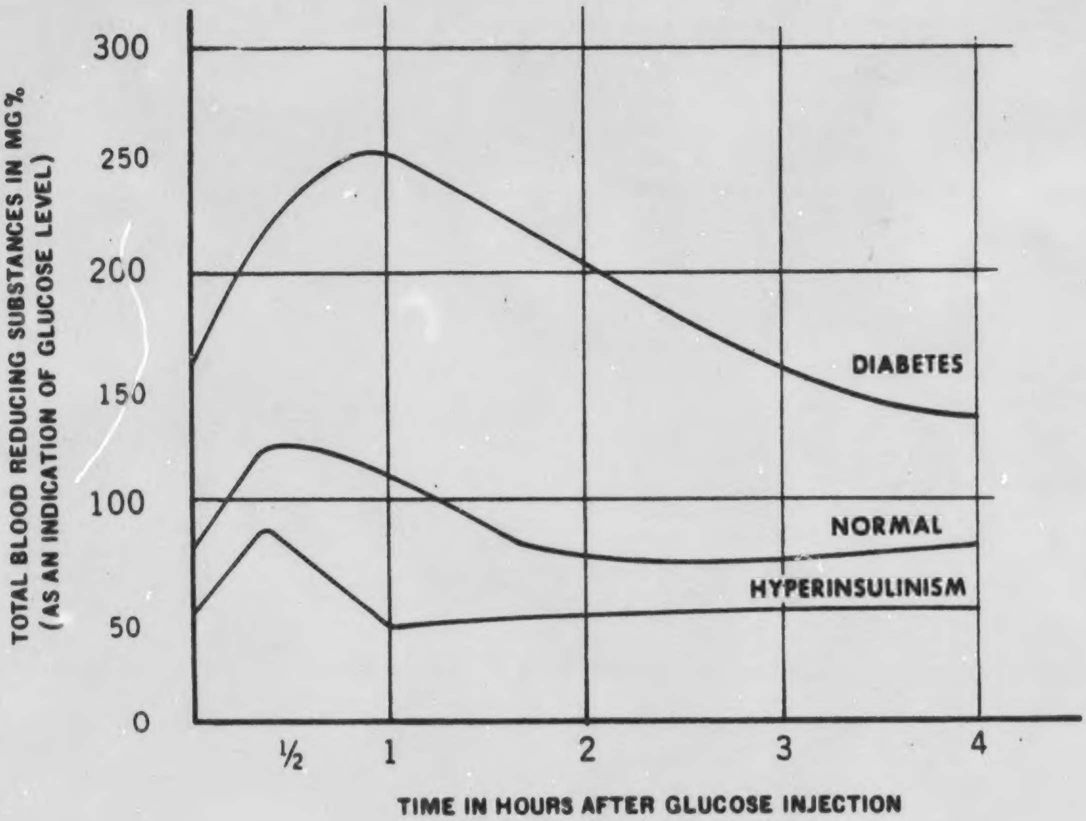

Figure 12. Glucose tolerance curves.

be found in hypothyroidism, hepatic disease, Addison's disease, and hyperinsulinism. Some individuals have a low renal threshold for glucose; and hence, glucose may appear in the urine (glycosuria) in the absence of a pathological condition. This condition is frequently described as renal diabetes.

8. Laboratory Tests for Glucose

8-1. As a laboratory technician, your most immediate concern is performance of laboratory tests. This section outlines various tests for glucose with a brief analysis of their relative merits.

8-2. **Folin-Wu Procedure.** In the Folin-Wu method, a protein-free filtrate is heated with an alkaline copper solution. The reduction of cupric hydroxide produces cuprous oxide. Phosphomolybdic acid is then reduced to molybdenum blue, which has an uncertain chemical composition. This blue color is compared with that of a glucose standard which is available through medical supply channels. If used with a tungstate filtrate, the Folin-Wu method does not indicate the true glucose level of blood. Besides other reducing sugars, the procedure will detect glutathione, uric acid, ascorbic acid, and some amino acids. If used with a zinc (Somogyi) filtrate, the Folin-Wu procedure is quite specific for glucose. Many, if not most, laboratories have abandoned this procedure for methods more specific for glucose. This is a wise decision, since the error due to nonglucose reducing substances can be substantial.

8-3. **Ortho-Toluidine Procedure.** There is considerable controversy over the reliability of the o-toluidine method for glucose determinations. In principle, color development is based on the reaction between glucose in a TCA or STP filtrate and o-toluidine in glacial acetic acid to form a glycosylamine. Described in some detail by Dr. K.M. Dubowski in the journal "Clinical Chemistry," June 1962, this method is similar in principle to a rapid method for aldosaccharide determinations, described by Dr. Eric Hultman in "Nature," January 1959. Ortho-toluidine, as shown in figure 13, is only one of many aromatic amines which yields a color complex with glucose. For various reasons, o-toluidine was selected as the most suitable.[15]

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[15] Some of the other amines used were subsequently found to be carcinogenic.

MODIFICATIONS

Figure 13 of this publication has(have) been deleted in adapting this material for inclusion in the "Trial Implementation of a Model System to Provide Military Curriculum Materials for Use in Vocational and Technical Education." Deleted materials are copyrighted and could not be duplicated for use in vocational and technical education.

8-4. As pointed out in the earlier article by Hultman, the reaction is very sensitive to water, which according to Hultman should not exceed a final concentration of 10 percent. In most clinical procedures employing ortho-toluidine, the concentration does exceed 10 percent.

8-5. A second problem, apart from reducing the concentration of water, is encountered in preparing suitable o-toluidine reagent. Unless pure o-toluidine "from nitrate" is used, technical grade reagent must be distilled. The color should be yellow after the reagent has aged a few days. If stored in the dark, this reagent is quite stable. Quite recently an o-toluidine procedure became available which produces a more stable reaction than the original o-toluidine procedure described above. Thiourea is used as a stabilizing agent and some of the other difficulties are minimized because a semimicro amount of specimen is used. The value of this particular o-toluidine adaptation had not been evaluated sufficiently at the time this course was prepared to justify a conclusion.

8-6. A third problem with this procedure is the critical nature of the heating time. According to published appraisals of this method, heating for longer than 8 minutes causes loss of color. Further, there is some doubt as to the stability of color at room temperature. Careful timing and rapid cooling can offset this problem.

8-7. Because of its simplicity and near specificity for glucose, the o-toluidine procedure may be preferred by some laboratories despite potential difficulties. The preference should be a rational one, based on a quality control program. If results are consistently achieved within confidence limits, this would appear to be a satisfactory procedure. If not, the particular laboratory should carefully reevaluate the method it has selected for the performance of glucose tests. Values obtained by this method are comparable to those obtained by other "true glucose" methods.

8-8. **Enzymatic Procedures.** Enzymatic procedures are the most specific of the methods available to assay glucose. To perform these procedures you may use either a mixture of enzymes or a single enzyme. Yeast had been widely used in this connection until the advent of purified enzymes. One such purified enzyme is glucose oxidase, which converts glucose to gluconic acid and hydrogen peroxide. The hydrogen peroxide is used as the key to color development in most enzyme procedures by reacting it with a compound such as o-dianisidine to form a color. The color reaction may be diagrammed as follows:

$$\text{Glucose} + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{dye} - H \xrightarrow{\text{peroxidase}} \text{Color} + H_2O$$

8-9. Glucose oxidase is specific for one isomer of glucose (Beta-D-glucose) and it may, therefore, be necessary to incorporate another enzyme in the reaction which induces a shift from the nonutilizable alpha isomer present to the beta form which can be oxidized. The shift is termed "mutarotation," and is essential to insure complete assay of the glucose. About 35 percent of blood glucose is in the nonusable, or alpha, form. Various commercial preparations are available in the form of reagents or tapes which are enzymatic in principle. Further, the principle has been applied to automatic equipment. Enzyme methods are widely accepted as reliable for the assay of glucose. Again, as with all procedures, reliability should be determined within the context of a quality control program.

8-10. **Somogyi-Nelson True Glucose.** Alkaline copper solution is heated with a filtrate of the specimen which causes cupric hydroxide to be reduced to cuprous oxide. Arsenomolybdate is then added and reduced to form a complex which is a deep green-blue color. This color is proportional to the amount of reducing sugar present. A Somogyi filtrate consists of 1 part blood, 5 parts water, 2 parts 0.3*N* barium hydroxide, and 2 parts 5 percent zinc sulfate ($ZnSO_4 \cdot 7H_2O$). The use of a zinc filtrate may interfere with copper reduction when micro procedures are used.

8-11. **Ferricyanide Reaction.** Several ferricyanide methods have been used to determine glucose. The reactions depend upon the reduction in an alkaline solution of yellow ferricyanide ions, $Fe(CN)_6^{---}$, to colorless ferrocyanide ions, $Fe(CN)_6^{----}$, by glucose. This technique has gained interest since its institution as an automated method by the use of an Autoanalyzer. A serum dialysate is mixed with alkaline ferricyanide in a continuous stream. It is then sent through a glass delay coil maintained at 95° C. The ferricyanide is reduced to a relatively colorless ferrocyanide dependent upon glucose concentration. The reacted stream goes through a flow cuvette, where the loss in color is read spectrophotometrically and recorded before the stream goes to waste. This Autoanalyzer procedure is outlined in figure 14.

8-12. Several aspects of automation may need clarification at this point. A dialysate is the material which diffuses (or dialyzes) through a membrane. In this glucose method, dialysis is used to render the specimen protein-free. This process occurs at a constant rate as the diluted serum sample (specimen stream) is pumped through the dialyzer module on one side of the membrane. You can see from figure 14 that alkaline ferricyanide reagent (reagent stream) also passes through the dialyzer. The reagent stream passes through on the opposite side of the dialyzing membrane from the sample and carries sample dialysate along with it. The specimen stream goes to waste from the dialyzer. The reagent stream, containing dialysate, continues on to the 95° C. heating bath where reduction of ferricyanide occurs. Transport tubing carries the reacted stream to a colorimeter where the stream is debubbled before passing into a "flow-through" cuvette. The optical density is automatically recorded on a graph paper strip. This complete process will quantitate 60 samples per hour. Both glucose control serum and standards are included in each run for standardization and quality control. Other concepts involving automated analytical methods will be discussed as they develop in this text.

8-13. **Other Glucose Procedures.** Electrophoresis and paper chromatography are both used for the determination of glucose, although neither will be further discussed in this course in connection with glucose. Various reduction reactions besides those of copper and ferricyanide are also used, including the reduction of bismuth and the reduction of potassium permanganate. One reaction which is specific for sugars uses phenol in a solution of aqueous methyl salicylate. This is the so-called PMS reagent available commercially. Protein, particularly albumin, interferes with the reaction, elevating the results. A small amount of serum, urine, or spinal fluid is generally used rather than whole blood. Chemically, PMS reagent functions through combination of the phenolic hydroxl group with the aldehydic ending of the sugar molecule. There are also many other glucose procedures which are not discussed here. It might be added that with most methods for determining sugar, deproteinization is responsible for the greatest difference in values obtained.

8-14. **Glucose Tolerance Tests.** In the usual

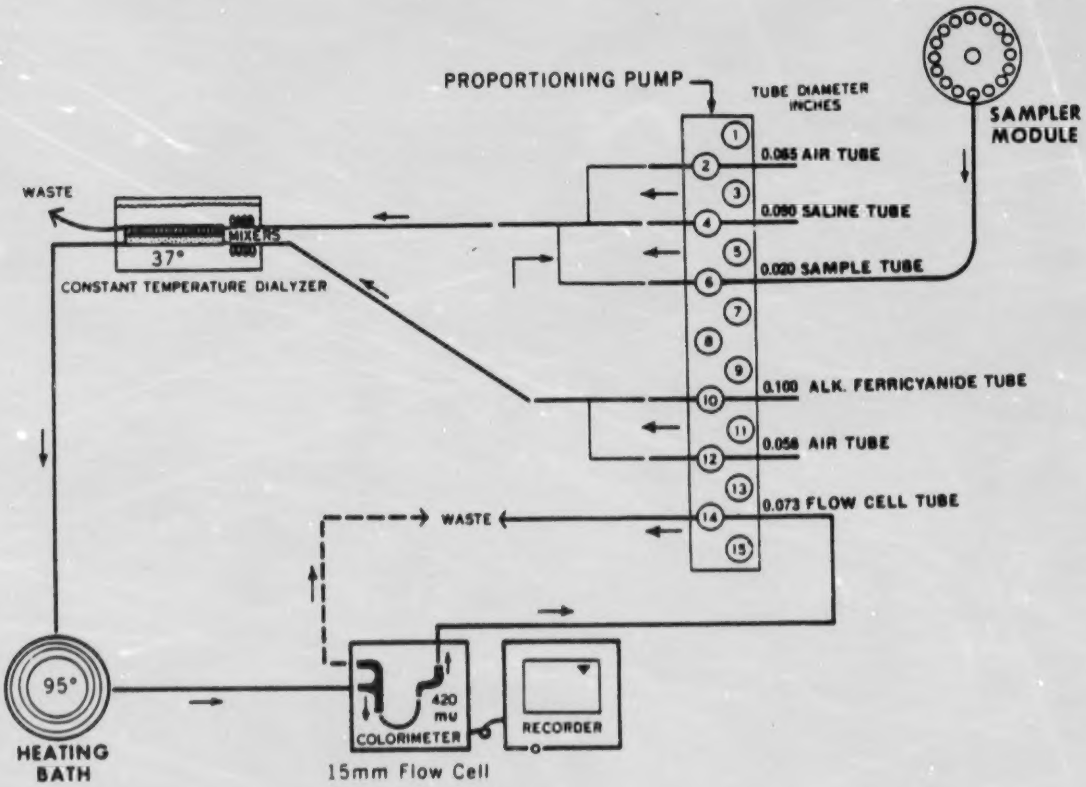
PROPORTIONING PUMP
TUBE DIAMETER INCHES
SAMPLER MODULE
WASTE
MIXERS
37°
CONSTANT TEMPERATURE DIALYZER
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0.065 AIR TUBE
0.090 SALINE TUBE
0.020 SAMPLE TUBE
0.100 ALK. FERRICYANIDE TUBE
0.056 AIR TUBE
0.073 FLOW CELL TUBE
WASTE
95°
HEATING BATH
420 mu
COLORIMETER
RECORDER
15mm Flow Cell

Figure 14. Ferricyanide method for glucose.

glucose tolerance procedure, a measured amount of glucose is administered orally, and at periodic intervals blood and urine samples are analyzed for glucose and ketones. The amount of glucose administered is generally 50 or 100 g for an adult. There is no evidence to suggest that the amount of glucose administered must be exact as long as uniformity is maintained. Glucose, which is available through supply channels, can be weighed on a laboratory balance. Flavored commercial preparations may be used in place of those available through supply channels.

8-15. The test begins with a fasting blood sample and the patient should not eat during the test. Further, vigorous exercise should be avoided, as this reduces the blood glucose level. A patient may become restless during the hourly intervals and use this time to rush about the base or do other physical work. He should be cautioned to refrain from such activity. There is no objection to the patient drinking moderate amounts of water during the test if he desires to do so. Some authorities believe that an oral glucose tolerance test leaves much to be desired and should be replaced by the IV tolerance. This would appear to be a matter of clinical judgment based on practical considerations and needs of the particular patient. In many cases a 2-hour postprandial specimen could be used to attain the medical objective, but again, this would be for the physician to decide. The intent of a 2-hour postprandial specimen is to measure the blood glucose level 2 hours after an adequate carbohydrate meal, usually to aid in the diagnosis of diabetes.

8-16. Since most tolerance tests are done in the morning to meet laboratory schedules, the meal may be less than adequate. Breakfast means different things to various people, ranging from dry toast to a three-course meal. Therefore, it is better to insure adequate intake by supplying 50 to 100 g of glucose. Further, it insures conformity to a schedule and reduces physical exercise of the patient, who might travel some distance between the laboratory and a place to eat. A fasting specimen followed by a 2-hour postprandial specimen is an expedient which has replaced many of the lengthy glucose tolerance tests performed in U.S. Air Force medical facilities and elsewhere. As noted in the preceding section, figure 12 shows the normal and a few abnormal responses to glucose intake. A detailed discussion of particular glucose tolerance curves, together with associated data, can be found in the medical literature, and is not usually a primary concern of the laboratory.

CHAPTER 5

Enzymes

THE FIRST enzymologists were also the world's first biochemists. They were people who studied the chemical changes brought about by yeast, particularly with reference to the production of alcohol from sugars. The mechanism of alcohol production involving yeast was explained in some detail by Lavoisier in the 18th century, but the active component of yeast was not extracted and identified until just before the turn of the present century. This substance became known as an enzyme, which means "to leaven." Still the chemistry of biocatalysts (or enzymes) made only limited progress until 1926, when James B. Sumner crystallized the enzyme urease from the jackbean plant. In the decade which followed, Warburg and others were credited with crystallizing a number of purified enzymes.

2. It should be made clear that all of the progress in enzyme chemistry did not rest upon purification and crystallization of specific enzymes. Many investigators studied particular enzymes in their more or less natural cellular environment through the control of interfering or unrelated metabolic activities of the cell. Today, many of the enzyme studies in hospital laboratories are pursued along such biological lines through the study of enzyme systems and of mixtures of enzymes in serum, whole blood, etc. Because of its proven worth, the rapidly developing field of enzyme chemistry has taken over a significant share of clinical chemistry. As will be discussed more fully, enzymes control an almost infinite number of chemical reactions in the body. Yet, each enzyme is specific for the reaction it catalyzes and can, therefore, usually be related to a particular body function. Although many other chemistry procedures will always be essential, enzyme studies are gaining increasing attention and usefulness.

9. Enzyme Chemistry

9-1. Enzyme chemistry is the study of biocatalysts, and is the most rapidly developing area of clinical chemistry. In this section, we attempt to answer two basic questions: (1) What are enzymes? and (2) How do they act? In order to study enzymes in the laboratory, you must control all of the factors that affect their activity. These are listed for you. Because of the relatively low concentration of enzymes in body fluids, enzymes are reported in arbitrary units of activity under specified conditions. In reporting enzyme concentrations, you should always identify the type of units expressed. In the final paragraph of this section, you will find a few words about nomenclature peculiar to enzymes. More complete rules of nomenclature are available from currently published information.[14]

9-2. **Chemical Nature of Enzymes.** Enzymes control all of the reactions that occur in the body. They are all protein in nature and are produced by various cells of the body. Because they control biochemical reactions, they are called biocatalysts. A catalyst is defined in chemistry as an agent which controls the speed of a reaction or maintains a reaction without itself reacting or undergoing change. There is some recent evidence, however, that enzymes may undergo some changes of structure when they catalyze biochemical reactions. Described as feedback inhibition, this process is thought to be one in which the enzyme molecule undergoes a rearrangement of subunits. Further, each enzyme is thought to exist in two alternative structural forms, a reactive and nonreactive state. As you may recall from the discussion of protein chemistry, the structure of a protein molecule is very complex. This places limitations on the structural analysis of enzymes with methods available for detecting changes in molecular arrangement or special configurations. For our purposes it is less important to analyze theoretical mechanisms than to recognize behavioral properties. An awareness, for example, that enzymes are extremely effective in low concentrations causes the clinical chemist to seek a particular way of reporting their presence and effectiveness. Conventional expressions of concentration such as mg–% would not prove to be a sensitive enough scale for the assay of enzymes. To overcome this problem, the biochemist has selected units of *activity*, and it is in terms of activity rather than concentration that enzymes are measured. Consequently, factors which affect enzyme activity must be known and controlled.

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[14] Recommendations of the 1964 International Union of Biochemistry on the "Nomenclature and Classification of Enzymes" are published in a paper-bound book. *Enzyme Nomenclature*, Elsevier Publishing Co., London, 1965.

9-3. **Factors Affecting Enzyme Activity.** There are many factors which affect enzyme activity. Among these are the enzyme concentration, temperature, the pH of the solution, radiation, inhibitors, and time. In the next few paragraphs, we will discuss each of these factors.

9-4. *Concentration.* The concentration of an enzyme will determine the rate of activity, although a curve plotted as concentration versus activity will not necessarily be linear. That is, activity varies with enzyme concentration, but is not directly proportional under all conditions and tends to reach a plateau as shown in figure 15. The very fact that an increase in concentration can be measured in the laboratory is useful, because the enzyme concentration in tissues and body fluids changes in certain clinical conditions. In most cases, it is the concentration of enzyme which is actually being measured when the technician expresses enzyme activity in arbitrary units. Likewise, concentration of the component upon which an enzyme acts to produce a chemical change will determine the activity expressed. The substance which undergoes the chemical change is called a *substrate*. Thus, we see that the concentration of substrate and the concentration of enzyme affect the activity of an enzyme.

9-5. *Temperature.* In general, the activity of any enzyme will approximately double for every 10° rise in temperature on the centigrade scale. Because enzymes are protein in nature, a temperature that is too high will inactivate the enzyme and even destroy it. Most human enzymes are denatured at 60° C. Consequently, enzymes are described as heat labile. It is also true that a decrease in temperature will retard the activity of many enzymes. Most enzymes have very little activity at low temperatures (a useful fact for the technician who wishes to store a sample of serum or other body fluid containing enzymes). Ice formation may inactivate an enzyme unless quick-freeze methods with dry ice or lyophilization (fast dry freezing) is used. In most clinical laboratories if an enzyme serum specimen must be stored for a short period of time, 5° C. is preferable to slow freezing. Of course, lyophilized specimens may be stored for many months. Some enzymes may continue to show significant activity at a decreased temperature. This is, incidentally, the primary reason why certain frozen foods should be treated with boiling water to inactivate the enzymes which cause them to deteriorate even at subzero temperatures. For the clinical laboratory worker, controlling temperatures is a constant concern. Enzyme studies are usually made in a water bath which is accurate within 1° C. An error of 3° could introduce an error of 30 percent with enzyme determinations routinely performed, at least in areas where the curve is linear.

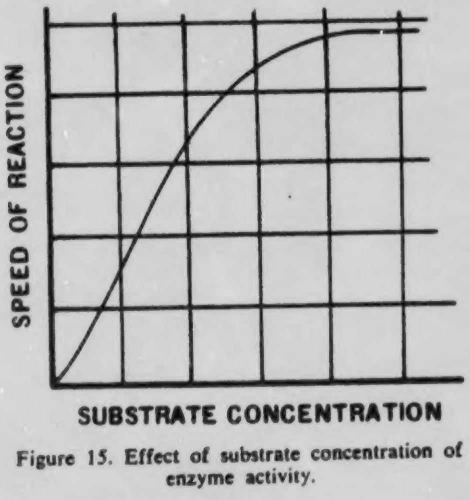

Figure 15. Effect of substrate concentration of enzyme activity.

9-6. *pH.* There is a specific pH value at which a particular enzyme is most active. In fact, enzymes are even characterized by the pH at which they are most active, as in the case of acid and alkaline phosphatase. It is a serious error indeed to report enzyme activity without controlling pH, because such an error could cause "normal" results where the activity is actually elevated. The use of carefully prepared reagents or commercial substrates precludes some of the difficulty, although the use of a pH meter and suitable buffers is the best technical approach.

9-7. *Radiation.* Forms of radiant energy, including ordinary sunlight and artificial light, can disrupt the protein molecules to the extent of affecting enzyme activity. Care must be taken in storing enzymes to avoid sources of radiant energy, particularly ultraviolet light.

9-8. *Inhibitors.* Glassware used in enzyme studies must be chemically clean, since the slightest contamination with heavy metals or certain salts will "poison" enzymes. Reagents such as mercuric nitrate, which is used in the Schales chloride procedure, or mercury from the Van Slyke apparatus, must be kept away from enzyme tests. The storage of reagents in metal containers (e.g., glycerine for urease suspension) should be avoided.

9-9. *Time of activity.* The period of time during which an enzyme acts will also determine the amount of substrate converted.

9-10. **Nomenclature.** Most of the enzymes first described do not follow any special system of naming. For example, salivary amylase was called ptyalin, from the Greek word for saliva. As enzyme chemistry became more conventional, there developed a system for naming enzymes which is in use today. A complete volume of rules for naming enzymes has been published recently as the result of an international agreement. It is sufficient for our purposes to recognize two of the basic systems commonly used.

a. Enzymes are sometimes named for the substrate upon which they act and are characterized by the ending *-ase.* Examples are phosphatase and amylase, which act on substrates containing phosphates and starch, respectively. (Amylum is Latin for starch.)

b. Enzymes may be named according to their function. An example would be transaminase, which functions in the transfer of an amino group. This appears to be the most meaningful method of naming in use today.

10. Clinically Significant Enzymes

10-1. Although there are probably hundreds of enzyme tests that can be performed, only the most practical determinations are included in this section. Of these, the most common are:

- *a.* Amylase, which is related most often to pancreatic disorders.
- *b.* Lipase, which is also for pancreatic function.
- *c.* Alkaline phosphatase test for liver and bone activity.
- *d.* Acid phosphatase, primarily for prostatic disorders.
- *e.* SGOT, for cardiac disturbances.
- *f.* SGPT, for liver disorders.
- *g.* LDH, also for cardiac disturbances.

As stated, there are many other tests, as well as many other reasons for doing the above tests besides the related disorders mentioned. We will also consider newer enzyme tests which have gained status in the clinical laboratory. Best known among these are leucine amino peptidase for various conditions, including carcinoma of the pancreas and 6-glucose phosphate dehydrogenase (6-GPD) to evaluate a hereditary deficency that results in induced hemolytic anemias. Later we will discuss seven lesser-known enzymes of importance.

10-2. **Amylase.** The enzyme hydrolyzes starch into the smaller units of dextrins and maltose. It is present in saliva and secretions of the pancreas. It is usually assayed in the clinical laboratory from serum or urine. Amylase is sometimes called diastase, and pancreatic amylase is sometimes called amylopsin. It is current practice to refrain from labeling amylase according to the area of the body in which it occurs and simply refer to this enzyme as amylase. This test is most commonly performed as a test for pancreatic function in patients complaining of abdominal pain.

10-3. There are various methods of assaying for amylase. The two methods most widely used measure either the amount of end product or the amount of starch remaining after activity. An example of the first technique is the Somogyi method, in which amylase is measured by the amount of reducing substances (calculated as mg per 100 ml plasma or serum) formed from the enzymatic hydrolysis of the starch contained in a starch substrate.

10-4. The second is the more popular method in Air Force laboratories and has been modified in many ways. In the Winslow modification, a known quantity of starch is added to serial dilutions of serum, as shown in figure 16, and incubated. The amylase present converts starch to sugar. After incubation, iodine solution is added and the tube containing the highest dilution of serum showing complete conversion to sugar (noted by absence of typical starch-iodine blue color) is taken as the endpoint. In the Carroway modification, the method is based on the measurement of a blue color and serial dilutions are not made. A control[17] tube is run which does not contain amylase and which is used as the basis for calculating the result in Carroway units as follows:

$$\frac{(\text{OD of control} - \text{OD of test})}{\text{OD of control}} \times 800 = \text{Carroway units}$$

One Carroway unit of amylase is defined as the amount of enzyme that will hydrolyze 10 mg of starch in 30 minutes to a stage at which no color is given by iodine. In a micro method, 1.0 ml of reagent containing 0.4 mg of starch is incubated 7.5 minutes with 0.02 ml of serum. This is equivalent to incubating 8,000 mg of starch with 100 ml of serum for 30 minutes. If all the starch were hydrolyzed, the serum amylase activity would be 8000/10, or 800 units per 100 ml. The factor of 800 is multiplied by the fraction of starch digested to give the units of amylase activity. Since the equation is valid for any photometer, no calibration is necessary. Normal values by the Carroway method are

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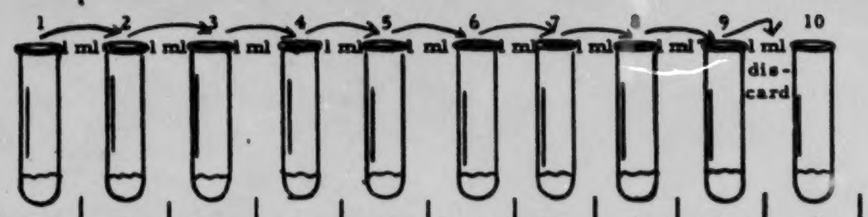
[17] Although widely accepted, the use of the term "control" in this instance is not altogether appropriate, because this tube does not control any aspect of the test. Rather, it serves as an arbitrary standard.



NaCl, 1 Percent (ml)	1	1	1	1	1	1	1	1	1	1
		1 milliliter of:								
Serum	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Dilution After Serum Transfer	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Starch, 0.1 Percent (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Iodine Solution (drops)	3	3	3	3	3	3	3	3	3	3
Titer (units)	4	8	16	32	64	128	256	512	1024	Control

Figure 16. Serum amylase dilution procedure (Winslow modification).

given as 60 to 160 Carroway units/100 ml of serum.

10-5. Procedures involving the starch and iodine reaction usually differ in normal values. As with all enzyme procedures, results should *always* be identified as to the kind of units, such as Winslow units, Carroway units, etc. It is frequently helpful to supply the physician with a list of normal values, or to indicate the normal values on the report form. The term "units" is by no means specific enough. Various hospitals perform enzyme procedures, such as the amylase test, which differ in normal values from a few units to hundreds of units. Few areas of clinical chemistry are in such need of standardization as are enzyme tests, and few enzyme tests enjoy the variety of procedures as the amylase test. In addition to carefully controlling all of the factors which affect enzyme activity, the technician must be very careful to maintain a suitable starch substrate. Improper preparation or contamination with either bacteria or mycotic organisms can render the starch substrate unsatisfactory. Keep in mind, also, that fluorides cannot be used to preserve specimens for enzyme studies.

10-6. **Lipase.** Although many laboratories no longer perform lipase tests, the ability of lipase to split neutral fats to form fatty acids and glycerol may be measured as a test for pancreatic function. In the usual procedure, serum is incubated with an olive oil emulsion substrate. Lipase activity results in splitting of the glyceryl-fatty acid ester bond with the liberation of free fatty acids. The amount of action is determined by titrating the liberated fatty acids with standard alkali, using thymolphthalein as the indicator. A glass electrode pH meter may also be used to detect the endpoint. After a set period of incubation, usually either 4 hours or 16 to 24 hours, the mixture is titrated with *N*/20 NaOH to a blue color with thymolphthalein (pH 10.6). The milliliters required to titrate the blank are subtracted from the milliliters required to titrate 1 ml of reacted serum to give the answer in lipase units. A 4-hour incubation should give normal values of 0.06 to 0.89 units, and a 16- to 24-hour incubation should give values of 0.20 to 1.50 units, but any value less than 2 units is generally considered normal. Certain correlations have been shown to exist between lipase and another enzyme, tributyrinase. Some investigators have substituted the assay of tributyrinase for the assay of lipase, but the relationship of the former to pancreatic function is less certain than lipase. Lipase levels of serum are known to show a significant decrease if the serum is hemolyzed, but postprandial or lipid serum has been shown to be quite valid.

10-7. **Phosphatases.** Increased alkaline phosphatase activity is often associated with liver dysfunction and bone diseases such as rickets. Acid phosphatase is elevated in certain conditions of the prostate. Other pathological conditions, including carcinoma of the breast and Paget's disease, may also be indicated by a rise in acid phosphatase activity. Alkaline phosphatase shows optimum activity at a substrate pH of 9.2 and 9.7, and acid phosphatase is optimum when the pH of the substrate is in the range of 4.9 to 5.1. Actually, measurement of alkaline or acid phosphatase activity involves the measurement of a group of phosphatase enzymes. For example, all of the acid phosphatase of serum is not from the prostate, but may be from the red blood cells or other sources. Further, these components differ somewhat in their chemical makeup.

10-8. The most widely used method of determining the activity of serum fractions collectively identified as acid or alkaline phosphatase is by the Shinowara, Jones, and Reinhart method. In this procedure serum is incubated with buffered glycerophosphate of a definite alkaline or acid pH for 1 hour at 37° C. The optimum pH of 5 for acid phosphatase is easily achieved with a buffer, regardless of variation in the amount of serum used. For alkaline phosphatase an optimum pH of 9.7 is achieved in the final substrate mixture with a buffer of pH 10.8; use of less serum than normally called for in the test requires compensation in the amount of alkaline buffer added. Otherwise, the pH of the final substrate mixture will be significantly higher than 9.7.

10-9. The difference between inorganic phosphate before and after incubation is an index of phosphatase activity as measured by the method of Fiske and Subbarow using molybdic and aminonaphthosulfonic acid reagents for color development. When this method is used, it is advisable to report the inorganic phosphorus level in addition to phosphatase activity.

Alkaline phosphatase
Adults: 2.2 to 8.6 mg inorganic phosphorus liberated per hour per 100 ml serum
Children: 3 to 14 mg inorganic phosphorus liberated per hour per 100 ml serum.
Acid phosphatase
0 to 2.0 mg inorganic phosphorus liberated per hour per 100 ml serum.

10-10. Another widely accepted method for phosphatases uses buffered p-nitrophenyl phosphate as a substrate. This compound yields p-nitrophenol upon hydrolysis as follows.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{PO_{4ase}} \text{p-nitrophenol} + H_3PO_4$$

The p-nitrophenyl becomes yellow upon the addition of NaOH, whereas p-nitrophenyl phosphate is colorless in either alkaline or acid solution. Intensity of the color is determined to a certain extent by the concentration of NaOH. Many substrates have been studied in connection with phosphatase activity. Some of the substrates are more specific for one type of phosphatase than for another. For example, it has been shown that alpha-naphthyl phosphate is twice as specific as beta-glycerophosphate for prostatic acid phosphatase.

10-11. A third method frequently uses reagents in the form of tablets. While the procedure is not as detailed as the one above, some laboratories use the tablets as a screening test. Alkaline phosphatase levels follow the rate of bone formation, which accounts for the higher level in children. Serum is the specimen of choice, since fluorides and oxalates both act as inhibitors. There is some question of specimen stability, but both alkaline and acid phosphatase are probably stable in the frozen state. It should be added that the residual phosphatase activity of milk is sometimes measured in U.S. Air Force laboratories to aid veterinary departments in evaluating the efficiency of milk pasteurization. The procedure is somewhat different from that used for serum, although the principle is usually the same.

10-12. **Transaminases.** The two principal transaminase enzymes for which blood is analyzed in the clinical laboratory are serum glutamicoxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT).

10-13. *SGOT.* This test is of greatest diagnostic value in cases of myocardial infarction. It is the more commonly performed of the two transaminase procedures discussed here. In theory, SGOT catalyzes the following biochemical reaction which takes place in tissues, including cardiac tissue:

$$\text{alpha-ketoglutarate} + \text{L-Asparate} \xrightarrow{SGOT} \text{glutamate} + \text{oxalacetate}$$

Many, if not most, laboratories use a commercially prepared substrate and color developer for this procedure because reagents which have not been stabilized are more sensitive to temperature fluctuations. This can be a source of significant error.

10-14. The various procedures available are not identical, some being much more involved than others. In the Umbreit method presented in AFM 160–49, *Laboratory Procedures in Clinical Chemistry,* the oxalacetic acid formed is reacted with dinitrophenylhydrazine to form a colored hydrazone which is measured spectrophotometrically. As with all enzyme tests, the units in which this test is reported depend upon conditions of the test. The normal values presented in AFM 160–49 (8 to 40 units) are equivalent to Frankel units. One Frankel unit of either SGO or SGP transaminase will form 4.82×10^{-4} micromoles of glutamate per minute at 25° C., and at pH of 7.5. Values are read from a nonlinear curve which is prepared from a series of standards. In preparation of the curve, the technician should follow instructions supplied by the manufacturer if a commercial "kit" is used. Unlike the procedure in AFM 160–49, some of the commercially prepared standards are not incubated at 37° C., a point which can be quite easily overlooked.

10-15. *SGPT*. Primarily a test for liver function, the procedure for glutamic-pyruvic transaminase measures the biocatalyst in the following reaction:

$$\text{alpha-ketoglutarate} + \text{L-alamine} \xrightarrow{\text{SGPT}} \text{glutamate} + \text{pyruvate}$$

The procedure and reagents are frequently identical with the SGOT procedure, except that a different substrate is used. Color development mixtures are interchangeable if the procedures are the same except for substrate. Normal values for serum SGPT levels given in AFM 160–49 (5 to 35 units) are equivalent to Frankel units for SGOT identified in the preceding paragraph. Cerebrospinal fluid values are normally lower for both SGOT and SGPT. It is also reported that some diagnostic importance can be attached to the ratio of serum SGOT to SGPT, the point being that SGPT values are higher than SGOT values only in cases of viral hepatitis. More important is the fact that a rise in transaminase values is of greater diagnostic value than a single determination. For example, a rise from 5 to 25 Frankel units may be notewothy even though neither value exceeds that established as normal. Although interpretation is the responsibility of the medical profession, it is a matter of concern for the technician to submit data which differentiates minimal but significant changes in the serum transaminase levels. Serum showing high transaminase values should be diluted and rerun. All of the factors outlined in the first part of this chapter must be considered as potential sources of error. Serum samples for SGPT studies are stable in the refrigerator for 2 to 3 days if the cells are separated, and samples for SGOT are stable somewhat longer. Storage time may be lengthened if the serum is frozen. In both tests, slight hemolysis will not elevate results beyond confidence limits, but very obvious hemolysis will produce elevated results.

10-16. **Lactic Dehydrogenase (LDH or LD).** This enzyme catalyzes the conversion of pyruvic acid to lactic acid, as well as the reverse reaction shown as follows:

$$\text{Pyruvic acid} + \text{beta-DPNH} \xleftrightarrow{\text{LDH}} \text{beta-DPN} + \text{lactic acid}$$

The term "DPN" stands for diphosphopyridine nucleotide, and "DPNH" for the reduced form of the nucleotide. The rate at which the concentration of DPNH decreases under conditions of the test is measured directly as a change in optical density with a spectrophotometer. This test has gained wide acceptance in the past few years and is now supplied in the form of prepared reagents through medical supply channels.

10-17. Lactic dehydrogenase is remarkably stable in serum separated from cells even at room temperature. However, refrigeration is recommended for an extended period of storage up to a week. Specimens to be stored for more than a week should be frozen. Hemolysis has a profound effect on this test, so hemolyzed serum must never be used. The report is rendered in arbitrary units, usually identified simply as LDH units. The normal is 60 to 100 LDH units per 100 ml of serum, although other normal ranges may be given, depending upon the procedure. The Sigma procedure, for instance, lists the normal total serum lactic dehydrogenase value as 100 to 350 units per ml. In this case, 1 unit is defined as "that which will cause a decrease of 0.001 unit per minute optical density at 340 mμ at 25° C. in a 3-ml reaction mixture, as measured through a 1-cm light path." The LDH level of serum rises in cases of myocardial infarction. LDH level of spinal fluid is elevated in certain conditions of the central nervous system.

10-18. **Leucine Amino-Peptidase (LAP).** Serum LAP is assayed to rule out or diagnose carcinoma of the pancreas and certain other conditions, including acute pancreatitis, malignancies, and liver disease. It is also elevated in the third trimester of pregnancy, and following some surgical procedures. Diagnosis frequently depends upon a comparison of urine and serum LDH values and upon the changes in serum LDH as the condition progresses. Nonmalignant causes tend to produce a more transient rise in serum LDH values. The enzyme LAP hydrolyzes certain leucine compounds and their derivatives. A common laboratory procedure uses a leucylnaphthylamide substrate proposed by Goldbarg and Rutenburg. The reaction is as follows:

$$\text{L-leucyl-beta-naphthylamide} + H_2O \xrightarrow{\text{LAP}} \text{Leucine} + \text{beta-naphthylamine}$$

The beta-naphthylamine which is formed is then assayed by the diazo reaction. LAP enzyme is reported to be stable in serum or urine up to 7 days at 4° C. and for several weeks if frozen.

10-19. The most common system of reporting is in G-R units established by Goldbarg and Rutenburg. Normal values are as follows:

	Serum	*24-Hour Urine*
Male	75 to 230 G-R units	50 to 175 G-R units
Female	80 to 210 G-R units	20 to 70 G-R units

Recently it has been shown that lactic dehydrogenase is composed of components or *isozymes* which can be separated by means of electrophoresis or chromatography. Both heat labile and heat stable fractions of LDH have been separated from serum and tentatively identified with liver disease and cardiac damage, respectively. It appears there is still much to be investigated in the resolution of enzyme components.

10-20. **Trypsin.** Trypsin levels are most commonly assayed from fecal or duodenal contents because only limited success has been reported in correlating the trypsin level of serum with clinical disorders. One situation in which a trypsin level may be helpful results when there is a decrease in duodenal enzymes due to obstruction. On the other hand, some of the other enzymes, particularly lipase, cannot be accurately assayed from fecal or duodenal contents. Various methods are used for trypsin analysis. In the method of Gross, casein (which is precipitated by acetic acid) is digested by trypsin, forming products which are not precipitated by acetic acid. The result is reported in terms of trypsin activity equivalent to the reciprocal of the number of milliliters of duodenal fluid in the first clear tube. A popular technique widely used in Air Force laboratories is to prepare a series of dilutions of a fecal specimen and then measure the capacity of trypsin to dissolve the gelatin of X-ray film. This can be accomplished with one centrifuge tube by placing 1 cc of specimen in the bottom of the tube with an applicator stick and then adding saline or water to the 10 mark with stirring. This represents a 1:10 dilution of specimen. A drop of suspension is transferred with an applicator stick to the emulsion side of undeveloped (green) X-ray film. A portion of the mixture is decanted from the centrifuge tube, a second dilution is made, and so on, until various drops have been transferred to the X-ray film, representing dilutions of 1:10, 1:20, etc., through 1:1280. The film is placed, in a covered Petri dish, in the incubator for 2 hours, removed, and washed in tapwater. The highest dilution showing trypsin activity is demonstrated by absence of the emulsion layer, visible as a clear zone. The film may be submitted as part of the lab report if desired. Other procedures are also used for trypsin activity, including the film test described in AFM 160–49.

10-21. **Glucose-6-Phosphate Dehydrogenase (G-6-PD).** This enzyme is presented as an example of an enzyme which has gained the attention of clinical laboratories in the past few years. Acute hemolytic anemias can be induced by the use of certain drugs in persons having a deficiency or decreased activity of glucose-6-phosphate dehyrogenase in their red blood cells. Many drugs are known to induce hemolysis, including primaquine drugs used in the treatment of malaria, various nitrofurans, some sulfonamides, acetophenetidin, and others. Individuals who develop hemolytic anemias frequently present a diagnostic problem as to the etiology of the anemia. It is extremely helpful to the physician if you are able to demonstrate an enzyme deficiency which can draw attention to the effect of drugs as a causative factor. Although less than 1 percent of the Caucasion population is believed to be lacking in this particular enzyme, the incidence is notably higher in Negroes, Sardinians, Asiatic Jews, Greeks, Turks, and other ethnic groups.[18] In a recent study among Nigerian newborns, an average of about 15 percent of the males and 11 percent of the females studied were found to be affected.[19] Statistics collected among American Negroes show an incidence of approximately 5 percent. This does not mean, of course, that all individuals having deficient G-6-PD develop hemolytic anemias. It is well established, however, that this defect is hereditary and predisposes the patient to developing a hemolytic anemia if treated with some of the drugs mentioned. Other substances known to induce hemolysis include fava beans, naphthalene, and acetanilid. A glucose-6-phosphate substrate is available commercially for the assay of this enzyme, although this is not yet routine in all clinical laboratories.

10-22. **Other Enzyme Procedures.** Other enzyme procedures which are currently available in kit form include the following:

a. Malic dehydrogenase (MDH)—It is believed that MDH levels follow a pattern similar to SGOT levels.

b. Sorbitol dehydrogenase (SDH)—This enzyme catalyzes the following reaction:

$$\text{D-fructose} + \text{beta-DPNH} \xrightarrow{\text{SDH}} \text{D-sorbitol} + \text{beta-DPNH}$$

Elevated levels of SDH have been reported in liver disease, myocardial infarction, and diabetes.

c. Alpha-hydroxybutyric dehydrogenase—This enzyme functions in the conversion of alpha-ketobutyric acid to alpha-hydroxybutyric acid. Elevated levels of this enzyme are usually found in conditions which result in elevated LDH levels. This procedure is also helpful in cases of muscular dystrophy and diseases of the pharynx (angina).

d. Phosphohexose isomerase—The enzyme phosphohexose isomerase catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate. Although not yet firmly established as a clinical procedure, the assay of this enzyme may aid in the diagnosis of carcinoma and viral hepatitis.

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[18] Kellermeyer, R. W., et al., "Hemolytic Effect of Therapeutic Drugs," *JAMA*, 180:388-394, May 5, 1962.

[19] Capps, F.P.A., et al., "Glucose-6-Phosphate Dehydrogenese Deficiency and Neonatal Jaundice in Nigeria," Lancet, 379-383, August 1963.

e. Ornithine carbamyl transferase (OCT)—Since this enzyme occurs almost exclusively in liver cells, elevation of OCT may be of diagnostic value in estimating liver cell damage. It catalyzes the conversion of citrulline and phosphate to ornithine and carbamyl phosphate.

f. Cholinesterase—The enzyme cholinesterase hydrolyzes various choline esters. It is of more or less diagnostic value in poisoning from organic phosphates which are present in certain insecticides and which cause a reduction in the serum cholinesterase activity.

g. Cytochrome oxidase—This enzyme is sometimes assayed from tissues as a histochemical procedure.

h. Isocitric dehydrogenase (ICD)—Reported to be of value in the diagnosis of liver cell damage, this enzyme catalyzes the following reaction:

$$\text{d-Isocritrate} + \text{TPN} \xrightarrow[\text{ICD}]{\text{Mn}++} \text{gamma-ketoglutarate} + CO_2 + \text{TPNH}$$

An intermediate product may be formed before CO_2 and TPNH. The ICD level may be elevated in a variety of diseases.

i. Aldolase—There is evidence that assay of aldolase levels may be of value in the diagnosis of muscular dystrophy, myocardial infarctions, and carcinoma of the prostate. Aldolase catalyzes the conversion of fructose-1, 6-diphosphate to dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate. It is to be expected that many other enzymes will be investigated in the near future, and that specific correlations will be drawn between their activities and metabolic or other clinical disorders.

j. Creatine phosphokinase (CPK)—Reported to be of value in the diagnosis of muscular dystrophy, myocardial infarction and hypothyroidism, CPK catalyses the following reaction.

$$\text{ATP} + \text{creatine} \overset{\text{CPK}}{\rightleftarrows} \text{ADP} + \text{phosphocreatine}$$

As with other enzyme procedures, the value of CPK units varies with the "kit" or procedure used. Purity of the substrate is very important for creatine phosphokinase, since the reaction is markedly affected by the presence of inorganic ions.

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No. 340–UV, MALIC DEHYDROGENASE in Serum at 340 mμ (Siegel-Bing Method).

No. 400, GLUCOSE-6-PHOSPHATE DEHYDROGENASE in Red Cells or Other Fluids.

No. 420, CHOLINESTERASE in Serum or Other Fluids at 400—440 m.

No. 495, α-HYDROXYBUTYRIC DEHYDROGENASE (αHBD) in Serum at approximately 500 mμ.

No. 500, LACTIC DEHYDROGENASE in Serum and Urine at approximately 400–550 mμ. (Berger-Broida Method).

No. 505, TRANSAMINASE, GO and GP, at approximately 490–530 mμ.

No. 520, CREATINE PHOSPHOKINASE in Serum (CPK) at 500–540 mμ.

No. 650, PHOSPHOHEXOSE ISOMERASE in Serum or Tissue Extracts at 470–510 mμ (Bodansky Method).

No. 661, CREATINE PHOSPHOKINASE in Serum (CPK) at 620–700 mμ.

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STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 200 through 210

Guide Numbers	
200	Introduction to Body Metabolites; Body Electrolytes: General; Nature of Electrolytes; Distribution of Electrolytes; Sodium; Potassium; Chloride; Determination of Chloride; pages 1-5
201	Body Electrolytes: Bicarbonate and Carbon Dioxide Content; Electrometric Measurement of pH; pages 5-8
202	Other Inorganic Metabolites; pages 8-11
203	Introduction to Liver Function Tests; Physiological Basis of Liver Function Tests; pages 12-15
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205	Introduction to Proteins; Chemistry and Physiology of Proteins; pages 19-21
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207	Introduction to Carbohydrates; Chemistry and Physiology of Carbohydrates; pages 25-30
208	Laboratory Test for Glucose; pages 30-33
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CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objective: To understand the significance of measuring body electrolytes and the clinical importance of calcium and phosphorus.

1. What is meant by Gibbs-Donnan equilibrium? (1-4)

2. Fluid moves into cells if the plasma is hypotonic. Why? (1-5,6)

3. What role do the kidneys have in maintaining electrolyte balance? (1-6)

4. How is it possible for the total ionic level of the plasma to diminish with no appreciable change in ionic concentration? (1-7)

5. What is the ion of greatest concentration in plasma? (1-8)

6. Name the principal cations of serum. (1-8, 9)

7. Why is it necessary to know the blood potassium level of a patient who receives IV infusions of saline? (1-9)

8. What is the ion most likely to diminish in gastric malabsorption syndromes? (1-11)

9. Why is a drop of nitric acid sometimes added to the serum before a direct serum chloride titration by the Schales method? (1-12)

10. What is the anion present in CSF in higher concentrations than in serum? (1-12)

11. What is the principle of bicarbonate titration? (1-16)

12. What does the symbolic term [H+] mean? (1-18)

13. How is the symbol for hydrogen, H, different from the symbol [H+]? (1-18)

14. What is the pCO_2 proportional to in the Henderson-Hasselbalch equation? (1-20)

15. Why must blood for CO_2 content or direct pH measurement be drawn anaerobically? (1-21)

16. What are the three unknowns necessary for a physician to establish the acid-base balance of his patient? (1-24)

17. What is meant by the *chloride shift?* (1-26)

18. In the chloride shift, by what is potassium balanced? (1-26)

19. If a patient loses CO_2 by hyperventilating, what results may be expected? (1-26)

20. What is the standard electrode which serves as the arbitrary basis for electro measurement of pH? Why is it important to know the type of electrode on a particular instrument? (1-28)

21. Of what is a calomel electrode composed? (1-29)

22. By what is overactivity of the parathyroid glands usually accompanied? (2-3)

23. In the sense of storing bodily energy, what element is called the powerhouse of the body? (2-6)

24. Describe the hydrolysis of ATP and explain the result. (2-6)

25. With what is the serum level of phosphorus closely associated? (2-7)

CHAPTER 2

Objective: To show an understanding of basic liver physiology and common tests for liver function.

1. What is the chief exocrine secretion of the liver? (3-2)

2. What are the primary functions of the gallbladder? (3-3)

3. Describe the physiological significance of bile. (3-3)

4. Why is it abnormal to find bilirubin in the feces? (3-4)

5. Why is bile green? (3-4)

6. How do you explain the fact that urine urobilinogen may increase in cases of obstruction where bilirubin available to the intestine for conversion to urobilinogen is diminished? (3-6)

7. What is the source of bile pigments? (3-7)

8. How is cholesterol formed and how is it excreted? (3-8)

9. How are cholesterol esters formed in the body? (3-9)

10. What percent of the body cholesterol is in the form of esters? (3-9)

11. Which of the forms of bilirubin discussed is/are soluble in methanol? (4-2)

12. List the essential components in the Lieberman-Burchard reaction. (4-12)

13. What may happen if wet glassware is used with acetic anhydride? (4-12)

14. What effect does bilirubin have on a cholesterol determination if the cholesterol is not extracted from the from the pigment? (4-13)

15. a. What is saponification?
    b. Why do some cholesterol procedures utilize saponification techniques? (4-17)

16. Why would the use of 0.962 *normal* barium sulfate instead of 0.962 *molar* barium sulfate result in thymol units which are higher by a factor of two? (4-20)

17. What is the standard for the thymol turbidity test? (4-20)

18. Which two factors in serum are most likely responsible for cephalin-cholesterol flocculation? (4-21)

19. How do serum lipids affect a cephalin-cholesterol flocculation test? (4-21)

20. Why is the BSP test referred to as a *retention* test rather than an *excretion* test? (4-23)

21. How does an elevated bilirubin affect the validity of a BSP test? (4-23)

CHAPTER 3

Objective: To show a knowledge of the chemistry and physiology of certain proteins and to be able to identify the common laboratory tests for proteins.

1. What is the basic structural unit of the protein molecule? (5-2)

2. What are polypeptides? (5-2)

3. Describe the protein molecule. (5-2)

4. Why are proteins described as amphoterous? (5-4)

5. Describe the forces which act to keep proteins in solution and explain how this relates to the isoelectric point. (5-4, 5)

6. In what units is the isoelectric point of proteins expressed? (5-5)

7. Describe the physical change of a specific protein in its optimal salt concentration at its electrically neutral pH. (5-5)

8. List the enzymes which function in protein digestion. (5-6)

9. Briefly describe the metabolism of proteins. (5-7, 8)

10. What happens to the ammonia produced by protein metabolism? (5-8)

11. What is meant by "transamination"? (5-8)

12. What causes PKU? (5-9)

13. What is meant by "essential" amino acids? (5-10)

14. What is the principle of the Gornall biuret method for total protein? (6-2)

15. What is the principle of the Kingsbury-Clark method for urine proteins? (6-4)

16. Why is trichloroacetic acid, instead of sulfosalicylic acid, used for CSF protein precipitation? (6-4)

17. a. What is the principal objection to the biuret method for CSF protein?
    b. What is the normal CSF protein level? (6-6)

18. What is the normal A/G ratio? (6-7)

19. Describe what happens if 28 percent sodium sulfite is added to an aqueous serum solution. Explain. (6-9)

20. Why is sodium sulfite recommended over sodium sulfate for the precipitation of serum globulins? (6-9)

21. How does the globulin result by the Howe technique, using 22.2 percent sodium sulfate, compare with results achieved with 28 percent sodium sulfite? (6-9)

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CHAPTER 4

Objectives: To demonstrate an understanding of the terminology of carbohydrate chemistry and the metabolism of carbohydrates; and how to conduct laboratory tests for glucose.

1. How do monosaccharides differ from disaccharides? (7-2)

2. Give the empirical formula for each of the following:
   a. monosaccharide.
   b. disaccharide
   c. polysaccharide
   (7-2)

3. Distinguish between dextrin and dextrose. (7-3)

4. What is a hexose? (7-3)

5. What causes *cis* and *trans* isomers? (7-4)

6. Define:
   a. isomers.
   b. optical isomers.
   c. polarized light.
   (7-4–6)

7. How does a (d)+ isomer differ from a (d)– isomer? (7-6)

8. How would you describe the optical activity of a racemic mixture with respect to polarized light? (7-6)

9. In what chemical form are carbonhydrates absorbed in the intestine? (7-9)

10. Discuss the major ways in which glucose is metabolized and distinguish between aerobic and anaerobic metabolism. (7-10)

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11. How is glucose principally stored in the body? (7-10)

12. What is the normal fasting true glucose level of blood and how is it physiologically maintained? (7-11)

13. Briefly describe a normal response to the ingestion of glucose. (7-15)

14. Define the following:
    a. diabetes mellitus.
    b. diabetes insipidus.
    c. renal diabetes.
    (7-12, 14, 16)

15. Name some substances other than glucose which contribute to the total reducing capacity of a whole blood PFF. (8-2)

16. *a.* What is the principle of the Folin-Wu glucose procedure?
    b. Does Folin-Wu yield true glucose values if used with an STA filtrate?
    (8-2)

17. What is the result of the action of glucose oxidase on glucose? (8-8)

18. Glucose oxidase is quite specific for beta-D-glucose. Of what practical consideration is this? (8-9)

19. What is meant by a postprandial glucose test? (8-15)

20. *a.* List some general arguments for a glucose tolerance test as opposed to a 2-hour postprandial.
    b. Cite some arguments against a glucose tolerance test as opposed to a postprandial blood sugar.
    (8-15, 16)

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CHAPTER 5

Objective: To show a knowledge of the nature of enzymes and the factors affecting their activity, with emphasis on the clinically significant enzymes.

1. What is the purpose of enzymes in the body? (Intro.)

2. List six factors which affect the activity of enzymes. (9-3)

3. How does the activity of an enzyme vary with its concentration? (9-4)

4. How significant is the temperature of a water bath in performing most enzyme procedures? (9-5)

5. How is the pH of an enzyme substrate maintained? (9-6)

6. Why would a specimen for transaminase activity deteriorate if placed by a window in direct sunlight for several hours? (9-7)

7. Give two ways in which enzymes are named. (9-10)

8. What is the chemical function of amylase? (10-2)

9. Explain the factor of 800 in the calculation for the Carroway micro procedure. (10-4)

10. A patient is transferred to your medical facility and the chart shows an amylase activity of 250 units. The physician asks you if this is normal. What would you reply? (10-5)

11. What is the chemical function of lipase? (10-6)

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12. What is the most common clinical reason for requesting acid phosphatase tests? (10-7)

13. Name the optimal pH of the substrate for determining
    a. acid phosphatase.
    b. alkaline phosphatase.
    (10-7)

14. A test for acid phosphatase is ordered on a female patient. What dysfunction may be indicated? (10-7)

15. What is the principle of the alkaline and acid phosphatase procedure by each of the following methods?
    a. Shinowara, Jones, and Reinhart.
    b. P-nitrophenyl.
    (10-8, 10)

16. A specimen is submitted in lithium oxalate for acid phosphatase. The patient cannot be recalled and you are asked if the plasma is suitable. What would you reply? (10-11)

17. What is the primary diagnostic value of
    a. SGOT?
    b. SGPT?
    (10-13, 15)

18. It is brought to your attention that SGOT values in your laboratory are nearly always higher than SGPT values when both tests are performed on the same patient. How would you evaluate this situation? (10-15)

19. *a.* How stable is LDH in serum which has been separated from the cells?
    b. Slightly hemolyzed serum is submitted for the following tests: SGOT, SGPT, LDH. What effect, if any, would the hemolysis have upon each of the three tests? (10-15, 17)

20. A request form is submitted for a serum LAP test. This test is not performed at your laboratory.
    a. How would you prepare the specimen for shipping?
    b. The results are returned from your consultant laboratory as 200 GR units. What does this mean?
    (10-18, 19)

ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. Gibbs-Donnan equilibrium is the balanced system of unequally distributed ions separated by a semipermeable membrane.

2. To establish osmotic equilibrium.

3. Kidneys conserve water and electrolytes that are lost in other ways, and excrete excesses.

4. The total plasma volume may also diminish.

5. Sodium.

6. Sodium and potassium.

7. As the patient's blood is diluted with potassium-deficient fluids, he may develop hypokalemia.

8. Chloride.

9. Nitric acid lowers pH and prevents formation of a color complex.

10. Chloride.

11. Serum or plasma is reacted with a known excess of acid; the acid is then back-titrated with standard NaOH which mathematically represents the original bicarbonate concentration.

12. The symbol for hydrogen, H, with the positive ionic sign, both inclosed in brackets means hydrogen ion concentration.

13. The symbol for hydrogen, H, without the positive sign means the neutral element hydrogen. However, with the charge symbol, H^+, it means hydrogen ions.

14. pCO_2 is proportional to carbonic acid or to the dissolved CO_2 in the Henderson-Hasselbalch equation.

15. Blood for CO_2 content or direct pH must be drawn anaerobically because CO_2 will diffuse to any phase such as air which has a lower pCO_2. If CO_2 diffused from the plasma it would, of course, change the CO_2 content and the direct pH value.

16. The Henderson-Hasselbalch equation has three unknowns, the patient's blood pH, bicarbonate, and carbonic acid concentrations. It can be balanced if any two of these is known and the third unknown is calculated

17. The shift which takes place between the plasma and the cell with respect to chloride and bicarbonate to maintain electrical neutrality.

18. An increase in bicarbonate.

19. An increase in chloride and a decrease in bicarbonate.

20. The hydrogen electrode is the basic standard electrode. It is important to know the nature of the electrode because each has characteristic capabilities and limitations and should be used to measure the pH of solutions for which it was designed.

21. Mercury.

22. An elevation in calcium.

23. Phosphorus.

24. The terminal phosphate is transferred from ATP to water to form ADP and H_3PO_4. The result is a group transfer potential or release of energy.

25. Calcium.

CHAPTER 2

1. Bile.

2. Store and concentrate bile.

3. Bile performs a digestive function in the emulsification of fats and activation of certain enzymes. It also functions in an excretory capacity, particularly with regard to cholesterol.

4. Bilirubin is normally reduced to urobilinogen in the intestines.

5. Bile is green due to biliverdin.

6. Although less urobilinogen is available for reabsorption in the intestines, liver damage may be severe enough to interfere with the uptake and transformation of urobilinogen in the liver. Hence, it appears in the urine in abnormal amounts.

7. Hemoglobin.

8. Cholesterol is formed from acetate ions in the liver and excreted as cholic acid in the bile.

9. Cholesterol esters are formed from organic acids in combination with the hydroxyl group of the cholesterol molecule.

10. 75 percent.

11. Both direct and indirect are soluble in methanol.

12. Cholesterol, acetic anhydride, glacial acetic acid, and sulfuric acid.

13. A violent reaction or explosion may result.

14. The cholesterol will be falsely elevated.

15. a. Saponification is the process by which alkalis react with lipids to produce soaps and free alcohols.
    b. Saponification is advantageous in the cholesterol procedure by converting the various forms of cholesterol to one which can be reliably extracted and reacted.

16. Since barium has a valence of +2, the 0.962 *normal* standard would be one-half as concentrated as a 0.962 *molar* solution. When used to calculate unknowns, the unknown would then appear twice as high as it would with 0.962 *molar* standard.

17. A suspension of barium sulfate is the standard.

18. An increase in gamma globulin, a decrease in albumin, or both.

19. Serum lipids have no appreciable effect.

20. The BSP test is called a retention test because BSP is reported in percent dye retained rather than in percent excreted.

21. A high bilirubin would invalidate a BSP test only if the jaundice is due to blockage of the bile passages (obstructive) since retention is in this case not necessarily due to liver cell damage. Photometric interference occurs above 20 mg-%.

CHAPTER 3

1. The basic structural unit of the protein molecule is an amino acid, designated as $R\text{-}CH\ (NH_2)\text{-}COOH$.

2. Polypeptides are chains of amino acids.

3. Proteins are chains of amino acids which vary in character and sequence. The amino acids are combined in polypeptide chains to form large molecules of high molecular weight.

4. Proteins act as both acids and bases and are therefore described as "amphoterous," which is Greek for "both."

5. Proteins are held in solution as emulsoid colloids under the influence of two opposing forces:
   a. The attraction of protein molecules for molecules of the solvent which favors suspension.
   b. The attraction of protein molecules for each other which favors precipitation.
   The isoelectric point is the pH at which force number *a* is overcome by force number *b*.

6. Isoelectric point is expressed in pH units.

7. At the electrically neutral pH of a specific protein (isoelectric point) in an optimal salt concentration, the protein fraction will precipitate or "salt out."

8. Pepsin, trypsin, chymotrypsis, peptidase, and carboxypeptidase.

9. Proteins are absorbed as amino acids which are almost immediately taken up by the tissues. Storage is in a deaminated or phosphorylated form in the liver. The structural organization of the amino acids into tissue and specialized protein substances is a dynamic process with constant buildup and breakdown. Protein may be used for energy if carbohydrate or fat sources are unavailable.

10. Most of the ammonia is converted to urea.

11. Transamination is the transfer of an amino group from one compound to another.

12. PKU is caused by a hereditary metabolic disorder which results in a buildup of phenylalanine in the body.

13. The term "essential" means that such amino acids cannot be synthesized by convention of other amino acids and are necessary to life processes.

14. The protein in serum is reacted with an alkaline copper sulfate, in the presence of ethylenediaminetetra-acetic acid, to form a blue-violet complex (biuret reaction).

15. Protein is precipitated with sulfosalicylic acid after clearing the urine of phosphates with acetic acid.

16. Trichloroacetic acid is less selective in the precipitation of albumin versus globulin.

17. *a.* At low concentrations of protein the biuret method lacks sensitivity.
    b. 15-45 mg-% or less.

18. (1.5:1 to 2.5:1).

19. The globulin precipitates from solution because the serum-salt concentration which results (26.9%) causes the precipitation of that protein fraction commonly identified as globulin.

20. Sodium sulfite results in a more accurate and complete precipitation of globulin which agrees with electrophoretic patterns. Second, Na_2SO_3 is easier to work with because of its higher saturation level.

21. The use of 22.2 percent Na_2SO_4 results in a lower globulin value and a higher albumin level due to precipitation of alpha globulins with the albumin.

CHAPTER 4

1. Monosaccharides cannot be hydrolyzed into simple sugars. Disaccharides yield two molecules of a simple sugar upon hydrolysis.

2. *a.* $C_nH_{2n}O_n$.
   b. $C_n(H_2O)_{n-1}$.
   c. $(C_6H_{10}O_5)_n$.

3. Dextrose is another name for d-glucose, a hexose . Dextrin is a polysaccharide.

4. Saccharides with the formula $C_6H_{12}O_6$ are classified as hexoses.

5. *Cis* and *trans* isomers are the results of geometrical configurations caused by some factor (such as double bond) which prevents free rotation of the atoms.

6. *a.* Isomers are forms of the same substance which differ in atomic arrangement.
   b. Optical isomers meet the definition of isomers and are additionally characterized by their rotational effect on polarized light.
   c. Polarized light is light energy which vibrates in only one plane.

7. The (d)+ form rotates polarized light to the right, whereas the (d)– rotates polarized light to the left.

8. Racemic mixtures internally compensate with respect to polarized light and, therefore, do not rotate the plane of polarization.

9. Carbohydrates are absorbed as monosaccharides.

10. Glucose may be used for energy by processes described as aerobic or anaerobic. In the former, glucose is oxidized to carbon dioxide and water. In anaerobic metabolism, the end product is lactic acid. In addition to energy production, glucose may be converted to glycogen, a form in which it is stored. A third metabolic pathway for glucose would be its conversion to other carbohydrate or noncarbohydrate substances in the body.

11. Glucose is stored principally in the liver as glycogen.

12. The normal fasting true glucose level of blood is 70-100 mg-%. It is maintained by the cynamic process of glucose formation and use under the action of factors such as insulin, adrenal and pituitary hormones, and thyroxin.

13. The half-hour level normally exceeds the fasting, but by less than 75 mg-%. The one-hour level should be less than the half-hour level and all urine specimens are negative for glucose.

14. *a.* Diabetes mellitus is a disorder of metabolism directly caused by pancreatic malfunction and, hence, low insulin levels. Primarily, the disease may be due to overactivity of the pituitary or adrenal glands, liver disease, or various other causes.
    b. Diabetes insipidus is caused directly by pituitary disorders due to a tumor, injury, etc., and is etiologically exclusive of glucose metabolism.
    c. Renal diabetes is characterized by glycosuria due to a low renal threshold for glucose.

15. Gluthathione, uric acid, ascorbic acid, certain drugs, lactose, certain amino acids, and many other reducing substances.

16. *a.* In the Folin-Wu method, a protein-free filtrate is heated with an alkaline copper solution. The cuprous oxide produced by the reaction of cupric hydroxide and glucose reduces phosphomolybdic acid to molybdenum blue. This blue color is compared with that of a glucose standard.
    b. No.

17. Glucose oxidase converts glucose to gluconic acid and hydrogen peroxide.

18. About 35 percent of blood glucose is present as alpha-D-glucose, and hence a result from the action of glucose oxidase alone would be low by about one-third if not offset by other enzymes in the reducing reagent.

19. A postprandial test is one performed after a meal.

20. *a.* In a 2-hour postprandial,
    (1) conditions of the test, such as patient activity, are better controlled.
    (2) the carbohydrate intake is assured to be adequate.
    (3) subtle responses can be detected which may be a clinical necessity.
    (4) several results are better than one result.
    b. Glucose tolerance tests are:
    (1) time consuming for the laboratory.
    (2) clinically unnecessary when the postprandial is adequate.
    (3) incovenient for the patient.
    (4) expensive in terms of time and reagents.

CHAPTER 5

1. Enzymes control all of the chemical reactions of the body.

2. Concentration of enzymes and substrate; temperature; pH, radiation, inhibitors; and time of activity.

3. The activity of any enzyme varies directly with its concentration to a point at which further increase in enzyme produces no noticeable change in the speed of the reaction.

4. A 1° fluctuation can introduce an error of 10 percent plus or minus.

5. The substrate is buffered.

6. In addition to the probability of bacterial contamination of the specimen the transaminase may be damaged by radiant energy.

7. Enzymes are sometimes named for the substrate or for the function they perform.

8. Amylase catalyzes the reaction by which starch is hydrolyzed into maltose and dextrins.

9. In the micro procedure, the effect of incubation is the same as if 8,000 mg of starch had been incubated with 100 ml of serum for 30 minutes. A Carroway unit is based on incubating 10 mg of starch for 30 minutes. Hence, 8000/10 gives a factor of 800.

10. It is impossible to say whether or not 250 units of amylase is normal unless the kind of units is specified.

11. Lipase activity results in splitting of the glyceryl-fatty acid bond to liberate free fatty acids.

12. Prostatitis or prostate involvement, usually carcinoma of the prostate.

13. *a.* Acid phosphatase–4.9–5.1.
    b. Alkaline phosphatase–9.2–9.7.

14. Carcinoma of the breast.

15. *a.* Serum is incubated with buffered glycerophosphate of a definite alkaline or acid pH for 1 hour at 37° C. The difference between inorganic phosphate before and after incubation is a measure of phosphatase activity.

    b. Buffered p-nitrophenyl phosphate yields p-nitrophenol upon hydrolysis. The p-nitrophenyl becomes yellow upon the addition of NaOH and can be assayed spectrophotometrically.

16. The plasma is not suitable. Oxalates inhibit phosphatase activity.

17. *a.* For myocardial infraction.
    b. For liver disorders.

18. This is to be expected; SGOT values are nearly always higher than SGPT values on a particular patient except in cases of viral hepatitis.

19. *a.* LDH is very stable for 7 days, with refrigeration.
    b. SGOT and SGPT, minimal effect. The serum specimen for LDH is unsuitable if any hemolysis is present.

20. *a.* Serum should be shipped at 0–4° C.
    b. The result means that the serum leucine amino peptidase level is 200 Goldbarg-Rutenburg units, which is on the upper limits of normal. Further interpretation is a matter for the physician.

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STOP– **1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.** **2. USE NUMBER 1 PENCIL.**

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VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed.* Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001) It is clinically important to maintain the pH of blood within the range of

   a. 6.80 to 7.20.
   b. 7.20 to 7.35.
   c. 7.35 to 7.45.
   d. 7.40 to 7.55.

2. (002) Gibbs-Donnan equilibrium is best expressed as the

   a. balanced system maintained between equally distributed ions.
   b. balance maintained between diffusible and nondiffusible components.
   c. exchange of materials across a cell membrane which is superpermeable in nature.
   d. balanced system of unequally distributed ions separated by a permeable membrane.

3. (003) The concentration of electrolytes in plasma may not change appreciably if their loss from the plasma is accompanied by a concomitant loss of

   a. ionic concentration.
   b. extracellular fluid.
   c. intracellular fluid.
   d. cellular components.

4. (003-004) In the presence of a nondiffusible protein anion, the concentration of the diffusible sodium cation is

   a. less than the diffusible chloride anion.
   b. the same as the diffusible chloride anion.
   c. greater than the diffusible chloride anion.
   d. less than the nondiffusible protein.

5. (005) Which of the following ratios is maintained in one of the blood buffer systems?

   a. 1:20 of bicarbonate to carbonic acid.
   b. 20:1 of carbonic acid to bicarbonate.
   c. 1:20 of carbon dioxide to carbonic acid.
   d. 20:1 of bicarbonate to carbonic acid.

6. (006-007) A patient with an abnormal pCO_2 (respiratory involvement) should have his acid-base problem evaluated on the basis of all of the following *except*

   a. CO_2 combining power.
   b. CO_2 content.
   c. pCO_2.
   d. pH.

7. (007) The purpose of the chloride shift is to

   a. assess acid-base equilibrium.
   b. maintain electrical neutrality.
   c. assess the blood-buffering capacity.
   d. change the ratio of carbonic acid in the plasma.

8. (008) The standard electrode upon which all others are based is the

   a. calomel.
   b. quinhydrone.
   c. glass.
   d. hydrogen.

9. (009) A common clinical symptom of calcium deficiency is

   a. tetanus.
   b. tetany.
   c. hyperparathyroidism.
   d. hypokalemia.

10. (010) The serum level of phosphorus is closely associated with

a. arsenate.
b. silicate.
c. calcium.
d. sulfate.

11. (011) If a whole blood specimen is allowed to remain at room temperature for 3 hours, the serum phosphorus level will be

a. significantly increased.
b. slightly increased.
c. decreased.
d. not appreciably affected.

12. (012) The gallbladder empties into the

a. jejunum.
c. duodenum.
c. ileum.
d. cecum.

13. (013) Which of the following is *not* found in the feces of a normal healthy individual?

a. Mesobilinogen.
b. Stercobilinogen.
c. Urobilinogen.
d. Bilirubin.

14. (014) In *complete* extrahepatic obstruction, you would expect an increase in

a. direct and indirect serum bilirubin.
b. urine urobilinogen.
c. direct reacting serum bilirubin only.
d. intestinal bile.

15. (014) Cholesterol is a sterol, characterized by the cyclic structure of the

a. open chain.
b. phenanthrene ring.
c. double bond.
d. tetracyclic ring.

16. (014) What percent of the cholesterol in the body is in the form of esters?

a. 25 percent.
b. 50 percent.
c. 55 percent.
d. 75 percent.

17. (015) Direct bilirubin can be distinguished from indirect by adding

a. sulfuric acid.
b. sodium carbonate.
c. alcohol.
d. acetic anhydride.

18. (016) A bilirubin standard should *not* be stored

a. in a lighted room.
b. at 5° C.
c. at 0° C.
d. in the dark.

19. (016) An explosion may result if water or wet glassware comes in contact with

a. concentrated sulfuric acid.
b. caustic soda.
c. acetic anhydride.
d. glacial acetic acid.

20. (017) The chemical process by which fats are converted into a soap and glycerol upon the addition of an alkali is called

a. extravasation.
b. esterification.
c. precipitation.
d. saponification.

21. (017-018) Use of a .0962N thymol turbidity standard instead of a .0962M standard will cause results to be in units

a. one-half those given in AFM 160-49.
b. twice those given in AFM 160-49.
c. the same as those given in AFM 160-49.
d. unrelated to those given in AFM 160-49.

22. (018) For every kilogram of body weight, the amount of BSP dye to inject is

a. 1 mg.
b. 5 mg.
c. 1 ml of 5 percent.
d. 5 ml of 5 percent.

23. (019) The general formula for an alpha amino acid is

a. $R-CH(NH_2)-COOH$.
b. CH_3CHO.
c. $(CH_3)_4NOH$.
d. ROH.

24. (020) One example of a *simple* protein is

a. mucoprotein.
b. globulin.
c. phosphoprotein.
d. hemoglobin.

25. (020) In an optimal salt concentration at its isoelectric point, a specific protein fraction will

a. migrate.
b. fractionate.
c. denature.
d. precipitate.

26. (020) What enzyme breaks down proteins in the stomach?

a. Peptidase.
b. Amylase.
c. Pepsin.
d. Trypsin.

27. (021) How many essential amino acids are there?

a. 4.
b. 8.
c. 18.
d. 20.

28. (021) A nucleic acid associated with factors of hereditary control is

a. DNA.
b. PKU.
c. HIAA.
d. RNA.

29. (022) In the biuret reaction, protein is reacted with alkaline

a. sodium sulfate.
b. sodium sulfite.
c. copper sulfite.
d. copper sulfate.

30. (022) The Pandy test for CSF globulin is best described as

a. unreliable.
b. unstable.
c. acceptable.
d. desirable.

31. (023) Which of the following represents the range of normal A/G ratios?

a. 1:2 to 1.5:2.
b. 1.2:2 to 1.8:2.
c. 1.5:1 to 2.5:1.
d. 1.2:1 to 2.8:1.

32. (023) The normal value for total protein per 100 ml of serum is

a. 4 to 6 mg.
b. 6 to 8 mg.
c. 4 to 6 g.
d. 6 to 8 g.

33. (023-024) In a total protein procedure, you use 0.4 ml of serum and 9.6 ml of 28 percent sodium sulfite. What is the percent concentration of sodium sulfite in the serum sulfite mixture?

a. 9.6 percent.
b. 23.5 percent.
c. 26.9 percent.
d. 28 percent.

34. (024) The molecular weight of hemoglobin in atomic mass units is

a. 6,450 amu.
b. 10,000 amu.
c. 64,500 amu.
d. 100,000 amu.

35. (025) *Monosaccharides* differ from *disaccharides* in that the monosaccharides

a. are classified according to the number of carbon atoms they contain.
b. yield two molecules of a simple sugar upon hydrolysis.
c. became polysaccharides upon hydrolysis.
d. do not hydrolyze into other sugars.

36. (025-026) Glucose is also called

a. dextrose.
b. dextrin.
c. dextran.
d. by all of the above names.

37. (026-027) The two common forms of stereoisomerism are

a. due to their structural atomic arrangement.
b. geometric and optical.
c. due to double bond and structural arrangement.
d. mirror images of each other.

38. (027-028) Polarized light is defined as light which

a. passes through a crystal.
b. consists exclusively of ultraviolet rays.
c. vibrates in only one plane.
d. correlates with optical rotation.

39. (028) In what chemical form are carbohydrates absorbed in the intestine?

a. Polysaccharides.
b. Disaccharides.
c. Glyceraldehyde.
d. Monosaccharides.

40. (028-029) Glycolysis takes place

a. only in the liver.
b. only in the muscle.
c. in both the liver and in the muscle.
d. in neither the muscle nor in the liver.

41. (029) What term may be used to designate the series of steps involved in the aerobic metabolism of glucose?

a. The Krebs cycle.
b. The citric acid cycle.
c. The TCA cycle.
d. Any of the preceding terms may be used.

42. (029) The true glucose level of blood is

a. 60 to 80 mg-%.
b. 70 to 100 mg-%.
c. 80 to 120 mg-%.
d. 90 to 130 mg-%.

43. (030) As applied to the measurement of blood glucose, the Folin-Wu procedure is

a. generally unacceptable.
b. the best method to use.
c. generally error-free.
d. too specific.

44. (031) In the glucose oxidase procedure, glucose is converted to gluconic acid and

a. hydrogen peroxide.
b. water.
c. peroxidase.
d. o-dianisidine.

45. (031) In the Somogyi-Nelson true glucose procedure, alkaline copper solution is heated with a specimen filtrate which

a. reduces cupric oxide.
b. reduces zinc hydroxide.
c. reduces cupric hydroxide.
d. oxidizes arsenomolybdate.

46. (032) The so-called PMS reagent for determining glucose contains a solution of

a. phenol in methyl salicylate.
b. phenol in methyl cellusolve.
c. phenylphthalein in methanol.
d. phenol, methanol, and salicylic acid.

47. (034) Enzymes are best described as

a. phospholipids.
b. biocatalysts.
c. mucoproteins.
d. nucleoproteins.

48. (035) A curve of *enzyme activity* plotted versus *enzyme concentration* is usually

a. nonlinear.
b. elliptical.
c. a graphic direct proportion.
d. cardioid.

49. (035) The activity of an enzyme will approximately double for every temperature increase on the centigrade scale of

a. 1°.
b. 2°.
c. 5°.
d. 10°.

50. (036) *Time of enzyme activity* determines the

a. amount of enzyme used.
b. amount of substrate converted.
c. clinical value of the result.
d. substrate concentration.

51. (037) If hemolyzed serum is used, lipase values are

a. elevated significantly.
b. elevated slightly.
c. decreased.
d. not appreciably affected.

52. (038) In the Shinowara, Jones, and Reinhart method for alkaline phosphatase, the serum is incubated with buffered

a. glycerophosphate.
b. phospholipid.
c. phosphatase.
d. nitrophosphate.

53. (038) In the reaction alpha-ketoglutarate + L–Asparate + glutamate + oxalacetate, the catalyzing enzyme is

a. SGOT.
b. SGPT.
c. LDH.
d. G-6-PD.

54. (039) If stored at 4° C., leucine amino-peptidase (LAP) is stable in urine for approximately

a. 1 hour.
b. 12 to 24 hours.
c. 7 days.
d. 30 days.

55. (040) Trypsin levels are usually determined from contents obtained from the

a. mouth.
b. stomach.
c. pancreas.
d. intestinal tract.

56. (040) Glucose–6–phosphate dehydrogenase is found principally in

a. red blood cells.
b. serum.
c. urine.
d. cerebrospinal fluid.

57. (040-041) Ornithine carbamyl transferase (OCT) occurs almost exclusively in cells of

a. heart tissue.
b. liver tissue.
c. striated muscle.
d. smooth muscle.

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CDC 90411

MEDICAL LABORATORY TECHNICIAN - CLINICAL CHEMISTRY AND URINALYSIS

(AFSC 90470)

Volume 3

Laboratory Procedures in Clinical Chemistry (Part II)

Extension Course Institute
Air University

Preface

BEFORE BEGINNING this volume you must have completed Volumes 1 and 2 of Career Development Course 90411. In the first two volumes you studied general principles related to clinical chemistry and several fundamental categories of routine laboratory tests.

In the present volume we will continue very much in the same way as in Volume 2. That is, categories of laboratory tests will be described with regard to physiological and chemical principles. The significant difference between Volume 2 and Volume 3 is that Volume 3 includes procedures which are performed more frequently in large facilities than in laboratories of Class C or below.

Chapter 1 of Volume 3 consists of two sections: "Retention and Excretion Tests" and "Clearance and Concentration—Dilution Tests." Considerable attention is devoted to the calculations involved in the various types of clearance tests. In Chapter 2, the subject of gastric analysis is discussed, which is somewhat unique in terms of the specimen involved and its relationship (or lack of it) to other areas of clinical chemistry. After this brief chapter, we discuss the more complicated special chemistry tests.

There are two sections in Chapter 3; the first is entitled "Referral Chemistries," and includes chromatography, electrophoresis, and other tests generally performed only by consultant centers. The second part of Chapter 3, Section 6 of the volume, is a treatment of hormones.

Concluding this volume is a chapter concerned with toxicology from the standpoint of both the Class A laboratory and the smaller hospital laboratory.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to Med Svc Sch (MSSTW/120) Sheppard AFB TX 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB, Alabama 36114, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

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CHAPTER 1

Kidney Function Tests

IT IS THE purpose of a kidney function test to evaluate one or possibly more of the following kidney functions: (1) maintenance of acid-base and electrolyte balance, (2) excretion of waste products of metabolism, (3) maintenance of osmotic equilibrium, and (4) excretion of foreign substances, e.g., dyes, poisons or drugs. To properly evaluate the functions of the kidney, you must understand its anatomy and physiology. As will be described in greater detail in Volume 4, the functional unit of the kidney is the nephron unit, which is composed of the glomerulus and accompanying tubules. This unit selectively filters the blood which passes through it in order to eliminate or retain certain substances in the blood. At the same time, selective reabsorption and secretory activity occurs in the tubules, including retention of most of the water which passes through the nephron unit.

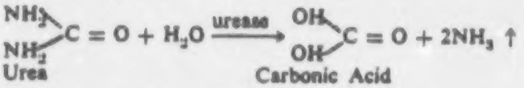
2. The ability of the kidneys to function properly depends upon three major factors. They are (1) the rate of renal blood flow, (2) the activity of the kidney tubules, and (3) the rate and efficiency of glomerular filtration. Accordingly, kidney function tests are sometimes classified to provide an indication as to the site of impaired kidney function. For example, a dye excretion test is valuable in measuring renal blood flow. Activity of the tubules can be measured with concentration and dilution tests, while the glomerular filtration rate can be evaluated by clearance tests. In addition to ordering a test to determine the origin or extent of kidney disorders, the physician may be interested in avoiding clinical problems which arise from kidney malfunction. These problems include a buildup of urea and other waste products in the blood, the concentration of which must be determined.

3. For the amount of time and effort required, few tests provide clinical information as definitive as kidney function tests. The tests which are most commonly performed in U.S. Air Force medical facilities are described in the following paragraphs. No attempt has been made to classify them according to a particular function of the kidney (e.g., tubular secretion) since some tests measure more than one function. You should be aware of the nature of kidney function tests from the standpoint of purpose, principle, and procedure. Four categories of kidney function tests which are of particular interest to the clinical laboratory are clearance tests, excretion tests, concentration-dilution tests, and retention tests.

1. Retention and Excretion Tests

1-1. Retention tests are a measurement of blood levels of such constituents as urea nitrogen to determine the extent to which they are being retained by the kidney. If we are trying to determine whether a substance is being eliminated from the blood, we perform an excretion test. For example, the PSP test is based on the excretion of a foreign substance (dye) by the kidney.

1-2. **Urea Nitrogen.** The blood urea nitrogen (BUN) test has almost completely replaced the test for nonprotein nitrogen (NPN) in most Air Force laboratories. This is a very common and extremely important laboratory procedure, but not necessarily a simple one. There are significant sources of error which can invalidate the results, especially if a good quality control program is not in effect. In the method of Gentzkow and Masen outlined in AFM 160-49, *Laboratory Procedures in Clinical Chemistry and Urinalysis*, urea in the specimen is hydrolyzed to ammonium carbonate by the enzyme urease and the protein is precipitated. The hydrolysis of urea may be illustrated as follows.



$$(NH_2)_2C=O + H_2O \xrightarrow{\text{urease}} (OH)_2C=O + 2NH_3 \uparrow$$

Urea Carbonic Acid

The ammonium carbonate produced is then nesslerized and compared with a suitable standard. Nessler's reagent is made up of the following reagents:

Mercuric iodide (HgI_2)	45.5 g.
Potassium iodide (KI)	34.9 g.
Potassium hydroxide (KOH), 10 M	200.0 ml.
Distilled water—q.s. to	1000.0 ml.

The primary difficulty with this procedure is color stability. Hence, some advantage may be gained by using a commercially prepared Nessler's reagent. The urease is easily prepared by suspending 1 g. of urease in 7 ml. of distilled water and adding 3 ml. of glycerin. The glycerin should come from a nonmetal container to avoid possible deactivation of the enzyme. The urease is usually stable but does become quite easily contaminated with mold. Commercial urease suspensions usually contain some type of mold inhibitor. Unsuitable urease can result in falsely decreased urea nitrogen values, as can unsuitable Nessler's reagent.

1-3. In the procedure for a BUN, the blood specimen is incubated with a few drops of urease suspension per milliliter of blood. It is obvious that ammonium oxalate must not be used as the anticoagulant for a BUN. It is convenient to use the same tube of blood for both BUN and glucose, as these two determinations are often requested simultaneously. The fact that blood collection tubes used for glucose may contain sodium fluoride should not interfere with the activity of urease. The fluoride concentration recommended is 1 to 2 mg. of sodium fluoride per milliliter of whole blood. This is sufficient to prevent the disappearance of glucose, but will not interfere with the subsequent use of urease. It has been reported that it would require 10 to 20 times that amount of fluoride to interfere with urease. Of course, if the blood is not to be used for glucose, there is no need to add the fluoride.

1-4. The time of incubation varies with the modification of method. Twenty minutes at room temperature or 15 minutes at 37° C. is quite adequate. Temperature is not critical, since this is a matter of complete conversion rather than a measurement of enzyme activity. The protein is then precipitated and the filtrate or centrifugate is reacted with Nessler's reagent. Sodium polyanetholsulfonate (SPS) is added as a color stabilizer, although 15 percent sodium citrate is reported to function as well or better in this capacity. The rate at which the Nessler's reagent is added and the length of time the color development mixture is allowed to stand before reading depends upon the activity of the specific Nessler's reagent a laboratory uses. This is not the type of procedure which lends itself to the use of a prepared curve unless commercially prepared reagents are used and special care is taken, since optical density readings of the standard are likely to vary with technique and reagents. High readings require repetition of the procedure by dilution of the protein free filtrate (PFF). The greatest problem is in securing high values which are within confidence limits. In fact, BUN values above 45 mg-% are not possible with some types of Nessler's reagent unless a dilution factor is applied.

1-5. Elevated urea nitrogen values are found in conditions associated with impaired renal function, particularly chronic nephritis. Cases of acute nephritis, cardiac failure, prostatic obstruction, and intestinal obstruction may also cause the urea nitrogen to be elevated. Lowered urea nitrogen results may be encountered in impaired liver function and certain other conditions. The laboratory area in which BUN procedures are performed should be free of ammonia fumes, because ammonia will elevate test results. Nessler's reagent must be kept away from the active urease suspension to avoid contamination of the urease before it has been allowed to catalyze the hydrolysis of urea. A Somogyi (zinc hydroxide) filtrate is considered the filtrate of choice, as it does not yield substances which produce interfering colors upon nesslerization. The error introduced by use of a tungstic acid filtrate, as suggested in AFM 160–49, may be of at least minor significance. The presence of reducing substances, including acetone, in the patient's serum can interfere by causing turbidity upon nesslerization. This is probably due to the formation of mercurous compounds from the mercury salts. Various techniques to remedy the occurrence of turbidity may be found in standard textbooks of clinical biochemical procedures. The normal range for a blood urea nitrogen is 10 to 18 mg. per 100 ml. of whole blood, serum, or plasma.

1-6. Other urea nitrogen procedures may be used. The reaction between ammonia and various color development mixtures other than mercuric iodide may be used by some laboratories. One method uses silver manganese nitrate for color development. Another procedure used with the autoanalyzer depends upon the dissociation of indophenol to produce a blue color. In principle, this is actually one of the earliest reactions for the assay of ammonia, having been originally described by Berthelot in 1859. Steps of the Berthelot reaction have been modified for present-day use, however. In addition to the procedures described, there are many other methods described in current literature. One of the procedures widely used and available as a stable commercial reagent consists of 2, 3, butanedione (diacetyl) monoxime in a buffered phosphate solution. This method is currently recommended for use in autoanalyzer methodology. Various methods available differ basically in that some measure ammonia and others measure urea directly.

1-7. **Uric Acid.** Occurring as the end product of purine metabolism, uric acid ($C_5H_4N_4O_3$) is a component of the total nonprotein nitrogen of the body. However, the blood uric acid level does not relate directly to the NPN level. Uric acid is normally excreted in the urine. The blood plasma level of uric acid depends upon both its rate of formation and the rate at which the kidneys are able to eliminate uric acid. Hence, elevated plasma uric acid levels are usually due to overproduction, decreased destruction, or decreased excretion. Diseases which contribute to one or more of these factors and which result in elevated levels include renal disorders, blood dyscrasias, lead poisoning, liver disease, and gout. Gout is a metabolic disease involving purine metabolism characterized by marked inflammation of the joints. The plasma uric acid level may rise to 10 or 15 mg-% during the acute phase of gout. Levels near the upper limit of normal, i.e. 6 to 8 mg-%, may be particularly difficult to interpret in view of technical difficulties sometimes encountered with the uric acid procedure. A quality control program is very helpful in this regard. Decreased values are of no known significance.

1-8. One of the most common procedures (Henry, et al.), for the determination of uric acid is as follows: Hexavalent phosphotungstic acid is reduced by uric acid to a lower valence in alkaline solution with the formation of a blue color. Sodium carbonate may be used as the source of alkali. The phosphotungstic acid reagent should be prepared in the following manner which differs from the method in AFM 160–49 before the 1967 revision.

Sodium tungstate (Na_2WO_4), R.G.	40 g.
Phosphoric acid (H_3PO_4), 85 percent	32 ml.
Lithium sulfate ($Li_2SO_4 \cdot H_2O$)	32 g.
Distilled water—q.s. to	1000 ml.

Dissolve the sodium tungstate in 300 ml. of distilled water in a reflux flask and add the phosphoric acid. Add several glass beads and reflux gently for 2 hours. Cool to room temperature and q.s. to 1000 ml. with distilled water. Add the lithium sulfate. NOTE: Refluxing is an essential part of reagent preparation.

Incorrect preparation of phosphotungstic acid reagent will probably result in turbidity of the color development mixture. Serum is the preferred specimen for analysis as it lacks the nonuric acid constituents and color-producing agents that may be found in whole blood. Normal values range from 4 to 6 mg. per 100 ml. serum. Determination of urine uric acid levels is of little practical importance, because the urine uric acid level varies with the intake of nucleoproteins.

1-9. **Creatinine.** One of the nonprotein constituents of blood, creatinine is also known chemically as methylglycocyamidine, $C_4H_7ON_3$. An elevation in the serum creatinine level *may* occur as a concomitant condition whenever the blood urea level is increased. This would be a likely occurrence in nephritis, urinary obstruction or suppression, cardiac decompensation, and intestinal obstruction. The assay of creatinine is of particular value when it is performed as a clearance test which will be discussed in a following paragraph. A widely accepted chemical procedure involves the Jaffe reaction. The creatinine in a PFF reacts with picric acid in an alkaline solution to form creatinine picrate which is a yellow to red color. A tungstic acid filtrate is suitable. There are a few precautions which must be observed. The specimen (serum or urine) should not contain dyes, e.g., PSP or BSP. Thymol and toluene are suitable urinary preservatives, but acids and alkalis must not be used because they convert creatine to creatinine. Be careful not to confuse creatinine with *creatine*. Creatine is described in a paragraph below.

1-10. The color development mixture should be read at room temperature because heat causes a deviation from Beer's law. Another precaution to be observed in addition to those already mentioned is in regard to the explosive nature of alkaline picrate. For this reason, alkaline picrate *must not* be heated in a dry-air oven to dehydrate it or for any other purpose. Alkaline picrate reagent is quite stable at room temperature if it is protected from light. It stains clothing and other objects and should, therefore, be handled carefully. The normal serum creatinine level is 1 to 2 mg. per 100 ml. of serum. It is current practice to apply a coefficient factor to creatinine determinations to allow for differences in body weight.

1-11. **Creatine.** Creatine is described chemically as methylglycocyamine NH : C (NH_2) N – (CH_3) CH_2COOH. Unlike creatinine, creatine is not a waste product of metabolism, but is derived from glycine, arginine, and methionine. Creatine functions in muscle contraction in the form of phosphocreatine. Creatine is assayed from urine by measuring an increase in creatinine concentration upon hydrolysis, and applying a conversion factor for differences in molecular weight. Hydrolysis is accomplished with the addition of acid and application of heat to the specimen by autoclaving. Increased values are found in fever, malnutrition, pregnancy, and diseases associated with muscular weakness. Creatine normals are as follows.

Urine: 0 to 200 mg. creatine excreted per 24-hour specimen.
Blood: 3 to 7 mg. per 100 ml. whole blood.

Normal values for creatine are somewhat controversial, particularly since as much as 10 to 30 percent of the urinary creatine may be converted to creatinine in the bladder.

1-12. **PSP Excretion Test.** Phenolsulfonphthalein (PSP, phenol red) is a dye which is readily removed from the blood and excreted by the normal, healthy kidney. Most of the dye is secreted by the kidney tubules while a lesser amount is filtered by the glomeruli. A small amount is excreted by the liver into the bile. The rate of excretion of PSP depends also on the state of renal blood flow. The amount of dye excreted is, therefore, an indication of the excretory state of the kidneys, especially the tubules.

1-13. PSP dye is supplied through medical supply channels in ampules containing 1 ml. of dye, which is equivalent to 6 mg. of PSP. This dye is transferred aseptically to a *sterile* syringe and injected intravenously by a physician. The laboratory technician frequently draws the dye into a syringe for use by the physician. It is good aseptic technique to attach a new sterile needle after the dye has been drawn into the syringe from the ampule and after air has been expelled from the syringe. It is also good aseptic technique to leave the protective sheath on the needle rather than place the sterile needle on a pad of cotton or gauze soaked with nonsterile alcohol. A potential source of error in this test is injecting too much dye and introducing an error in calculations. This is unlikely, however, since one would not normally use more than one ampule of dye per patient. The patient should drink water before the test. Whether the patient's bladder is empty or not at the beginning of the test is not particularly significant. Time of injection is noted and the patient is asked to empty his bladder completely at periods of exactly 15 minutes, 30 minutes, 1 hour, and 2 hours after injection of the dye. Collect and label all four specimens with the time of collection and volume of each specimen. Patients should be instructed not to discard any urine and should be provided with a container of adequate size or two regular size urine bottles. It is also advisable to note the volume on the report submitted to the physician. Turbid urine should be centrifuged or filtered before proceeding to the analysis.

1-14. The entire volume in each container may be transferred to correspondingly labeled 1 L. flasks and alkalized with 3 ml. of 4*N* NaOH and q.s.'d with water. An alternate procedure is to transfer 1 percent of the urine volume to a flask and add 1 ml. of 1 percent NaOH, followed by sufficient water to bring the volume to 10 ml. Actually, what this amounts to is reducing the volume of specimen by a factor of 100 and then reducing the dilution by a factor of 100. Either procedure accomplishes the same result. The normality of the NaOH need not be exact. Each specimen is then read in a spectrophotometer and compared with a standard or prepared graph. There is usually no significant variation in readings of the standard; and it is, therefore, quite acceptable to prepare a curve as outlined in AFM 160-49. The color is stable and there are only a few technical precautions to observe. First, the test should not be run within 24 hours of a BSP test because of slight interference. Second, bile and hemoglobin interfere, but either may be extracted from the urine with a zinc acetate-methanol mixture. Normal kidneys excrete at least 25 percent of the dye in 15 minutes and a total of at least 65 percent of the dye in 2 hours. The percentage of dye should diminish with each successive urine specimen and, of course, should not total more than 100 percent for all three specimens.

2. Clearance and Concentration-Dilution Tests

2-1. Calculations are presented in this section for expressing clearance in various ways. We assume that you are capable of performing and accurately calculating all of the tests mentioned. With reliable information provided by the laboratory, the physician is better able to diagnose and treat diseases which weigh heavily as far as the health and even the life of the patient are concerned. Clearance tests are designed, primarily, to measure the *efficiency* with which the kidney removes certain substances from the blood. The most common clearance tests employed in clinical diagnosis are the urea clearance and creatinine clearance tests. These techniques are used to evaluate kidney function by establishing a relationship between the rate of excretion of a test substance and the concentration of that substance in the blood or plasma. Concentration-dilution tests measure the capacity of the kidney tubules to maintain the specific gravity of urine within physiologically acceptable limits. The normal specific gravity of a random urine specimen is 1.003 to 1.030, and the normal specific gravity for a 24-hour urine specimen is 1.015 to 1.025.

2-2. **Urea Clearance Test.** In the urea clearance test, urea concentration of a diluted urine specimen, when compared to the blood urea concentration, is used as an index of the ability of the kidneys to remove nitrogenous substances from the blood. Although there is no need for elaborate preparation of the patient, a prescribed pattern should be followed to insure meaningful results. The patient need not fast, provided the meal is light. Diuretics such as coffee and tea must be avoided, as should vigorous exercise. Other details of the test may vary, with one possible routine described as follows. The patient is allowed about 20 minutes to drink 2 to 3 glasses of water. He is then allowed an additional half-hour to rest. At the end of the rest period, which may be extended, the patient *completely* empties his bladder and a clock timer is set for 30 minutes. At the end of exactly 30 minutes, blood and urine specimens are collected and the specimens are labeled. All of the urine is collected with complete emptying of the bladder and the clock is again set for 30 minutes. The patient drinks another glass of water. At the end of the second 30-minute interval, the patient again voids and collects all of his urine. Practically any given time interval may be used for this test. The urine volumes are then measured and the milliliters voided per minute are calculated based on the time interval of collection.

$$\text{ml./min.} = \frac{\text{volume of urine}}{\text{30 min.}}$$

Aliquots of the urine specimens are diluted according to table 1. The urea nitrogen levels of the blood and diluted urine specimens are determined by a reliable procedure, as described above.

2-3. Urea clearance depends upon the rate of urine formation. Since this is the case, correction factors may be included in calculating the dilution which will truly put the urine urea back into its original blood volume. A dilution table which compensates for variations in volume of urine output per minute as shown in table 1 may be used. It may not be apparent to you that the dilution table shown in table 1 as well as in AFM 160–49 is a 1:10 dilution of urine. Values reflect compensation for variations in urine output which would otherwise result in values inconsistent with the usual concept of clearance. If 1 ml. of this diluted urine is brought to a final volume of 10 ml. in the reaction mixture, the overall or final dilution of urine is 1:100. Consequently, the blood urea nitrogen procedure would also have to incorporate a 1:100 final dilution or allow for it mathematically. Most procedures call for 1 ml. of a 1:10 PFF brought to a final mixture volume of 10 ml. to result in a 1:100 dilution of blood. A second correction factor, labeled (K) in table 2, is used for variations in body size. Using the tables referred to, clearance is calculated for each urine specimen according to the formula shown below. Results of both specimens should fall within the same general range for the test to be valid.

$$\text{percent clearance} = \frac{U}{B} \times 100$$

U = urine urea nitrogen level
B = BUN level of a corresponding volume of blood

This is referred to as *average normal clearance*.

2-4. Problem Situation No. 1: A patient collects 60 ml. of urine within a 1-hour time interval. To what final volume would you dilute 1 ml. of this urine if you were performing an average normal urea clearance test?

2-5. Problem Situation No. 2: A child 5 feet in height produces 60 ml. of urine within a 1-hour time interval. Determine the volume of urine per minute in milliliters corrected for body size.

2-6. Solution to Problem Situation No. 1: Sixty milliliter in 60 minutes is 1 ml. per minute. Find 1 ml. in column V of table 1 and read the answer, 54 ml., from column 2.

2-7. Solution to Problem Situation No. 2: Five feet is equal to 152.4 cm. ($5 \times 12 \times 2.54 = 152.4$). Referring to table 2, find 150 in column 1 because this is nearest to 152.4; the correction factor (K) opposite 150 is 1.33; multiplying 1 ml. per min. by K, we arrive at 1.33 ml. per min. (1×1.33).

2-8. An alternative series of calculations preferred by many laboratories does not employ internal or hidden correction factors. Instead, the kind of calculation to be used depends upon the urine output. In this series, any convenient dilution of the urine may be made, provided the result is given in the same units of concentration as the blood urea level (e.g., mg./ml.). A dilution as shown in table 3 may be used to bring the urine urea concentration in line with blood levels. A correction factor for surface area variation is still used, since this is of particular importance in patients under 15 years of age. Terminology and corresponding calculations may be listed as follows.

a. Maximum clearance (C_m) describes a clearance in which the urine flow is greater than 2 ml./min. per 1.73 sq. meters of body surface. The formula for calculating maximum clearance is

$$C_m = \frac{UV}{B} \times K$$

where C_m = maximum clearance in ml. of blood/min.
U = urine urea nitrogen level.
B = BUN level of a corresponding volume of blood.
V = ml. of urine per minute, and

$$K = \frac{1.73}{\text{body surface area in square meters}}$$

K values are provided in table 2. Normal C_m values are 59 to 95 ml./min.

TABLE 1

UREA CLEARANCE DILUTION TABLE

Volume of urine per minute in ml. (V)	*Dilute 1 ml. of urine to:*	*Volume of urine per minute in ml. (V)*	*Dilute 1 ml. of urine to:*
0.50	76	2.00	37.5
0.52	75	2.10	35.7
0.54	73	2.20	34.1
0.56	72	2.30	32.6
0.58	71	2.40	31.2
0.60	70	2.50	30.0
0.62	69	2.60	28.8
0.64	68	2.70	27.8
0.66	67	2.80	26.8
0.68	66	2.90	25.8
0.70	65	3.00	25.0
0.72	64	3.10	24.2
0.74	63	3.20	23.4
0.76	62	3.30	22.7
0.78	61	3.40	22.0
0.80	60	3.50	21.4
0.82	60	3.60	20.8
0.84	59	3.70	20.3
0.86	58	3.80	19.7
0.88	58	3.90	19.2
0.90	57	4.00	18.7
0.92	56	4.10	18.3
0.94	56	4.20	17.9
0.96	55	4.30	17.4
0.98	55	4.40	17.0
1.00	54	4.50	16.7
1.05	53	4.60	16.3
1.10	51	4.70	16.0
1.15	50	4.80	15.6
1.20	49.3	4.90	15.3
1.25	48.3	5.00	15.0
1.30	47.4	5.20	14.4
1.35	46.5	5.40	13.9
1.40	45.6	5.60	13.4
1.45	44.8	5.80	12.9
1.50	44.1	6.00	12.5
1.55	43.4	6.20	12.1
1.60	42.7	6.40	11.7
1.65	42.1	6.60	11.4
1.70	40.8	6.80	11.0
1.80	40.3	7.00	10.7
1.85	39.7	7.20	10.4
1.90	39.2	7.40	10.1
1.95	38.5	7.60	9.9

b. Standard clearance (C_s) describes blood urea clearance in which the urine output is less than 2 ml./min. It is calculated as follows.

$$C_s = \frac{U\sqrt{VK}}{B}$$

where C_s = standard clearance in ml. of blood per minute,
U = urine urea nitrogen level,
B = BUN level of a corresponding volume of blood,
V = ml. of urine per minute, and
$K = \frac{1.73}{\text{body surface area in square meters}}$

K values are shown in table 2. Normal C_s values are 41 to 65 ml./min.

c. Minimal clearance is a concept employed where urine output is below 0.35 ml./min. The formula for minimal clearance is

$$\text{minimal clearance} = \frac{U}{B} \times 0.35$$

where U = urine urea nitrogen level and
B = BUN concentration expressed in the same terms as U.

The normal minimal clearance is 32 ml. of blood per minute. There have been many analytical treatises on the validity and meaning of the urea clearance test. Since space does not permit a complete discussion, it is adequate to merely recognize the wide latitude of variation in interpreting clearance tests. Remember that a clearance test is designed to measure the *efficiency* of an organ, in this case the kidney. This is a relatively difficult task under the best of circumstances.

TABLE 2

CORRECTION FACTOR *K* FOR UREA CLEARANCE TEST

Height	*Factor (K) to multiply V to obtain corrected V:*
Cm	
175	1.01
170	1.06
165	1.10
160	1.17
155	1.25
150	1.33
145	1.41
140	1.49
135	1.59
130	1.69
125	1.80
120	1.92
115	2.04
110	2.19
105	2.34
100	2.50
98	2.56
96	2.62
94	2.68
92	2.74
90	2.81
88	2.90
86	2.99
84	3.09
82	3.20
80	3.31
78	3.42
76	3.56
74	3.70
72	3.86

Cm = inches X 2.54.

TABLE 3

DILUTION TABLE FOR URINE SPECIMENS IN UREA CLEARANCE PROCEDURE

Vol (ml./min.)	*Dilution*
0.5 to 2.0	1:500
2.0 to 4.0	1:250
4.0 to 8.0	1:100
8.0 to 12.0	1:25

2-9. Problem Situation No. 3: Calculate maximum urea clearance (C_m) from the following data.

Urine output is 3 ml. per min.,
Blood urea nitrogen = 10 mg-%,
Urine urea nitrogen = 300 mg-%, and
Patient's height is 6 feet.

2-10. Problem Situation No. 4: Calculate standard urea clearance (C_s) of a child 100 cm. tall, given the following data.

Urine output is 1 ml. per min.,
Blood urea nitrogen = 0.5 mg. per ml.,
Urine urea nitrogen = 2 mg. per ml.

2-11. Problem Situation No. 5: Calculate minimal urea clearance from the following information.

Urine output = 0.2 ml. per min.,
Blood urea nitrogen = 0.2 mg. per ml., and
Urine urea nitrogen = 10 mg. per ml.

2-12. Solution to Problem Situation No. 3: The formula is

$$C_m = \frac{UV}{B} \times K$$

Values correspond as follows:

C_m = ?
U = 300 mg-%,
V = 3 ml. per min.,
B = 10 mg-%, and
K = 1(6 × 12 = 72"; 72 × 2.5 = 180 cm: Above 175 cm. no correction is required as noted from table 2.)

Then, $C_m = \frac{300 \times 3}{10} \times 1$
C_m = 90 ml. per minute.

2-13. Solution to Problem Situation No. 4: The formula for standard urea clearance (C_s) is:

$$C_s = \frac{U\sqrt{VK}}{B}$$

Values relate as follows:

C_s = ?
U = 2 mg. per ml.,
V = 1 ml. per min.,
B = 0.5 mg. per ml., and
K = 2.5 from table 2.

$$\text{Then, } C_s = \frac{2\sqrt{1 \times 2.5}}{0.5}$$

$$C_s = 2 \times \frac{1.6}{0.5}$$

C_s = 6 ml. per min. (Use slide rule or square root table if necessary.)

2-14. Solution to Problem Situation No. 5: Minimal urea clearance is determined as follows.

$$\text{Minimal clearance} = \frac{U}{B} \times 0.35,$$ the last figure being a constant.

U = 10 mg. per ml., and
B = 0.2 mg. per ml.

Then,

$$\text{minimal clearance} = \frac{10}{0.2} \times 0.35 = 17.5$$ or 18 ml. per min.

2-15. **Creatinine Clearance.** The clearance of creatinine from the blood is primarily an index of glomerular filtration rate. The test is performed in a manner similar to the urea clearance, with a few appropriate changes. The time period over which a urine specimen is collected is usually longer for the creatinine test, sometimes covering 24 hours. Further, protein intake of the patient should be controlled in the creatinine clearance. Calculations are performed as follows.

$$C = \frac{UV}{P} \times K$$

where, C = endogenous creatinine clearance,
U = mg./ml. urine creatinine,
V = ml. urine per minute,
P = mg./ml. plasma creatinine, and
K = 1.73 divided by patient's body surface area in square meters. (Refer to table 2.)

Normals range from 100 to 120 ml./min. or 116 to 148 L./24 hours. This test is sometimes compared with, or substituted for, the inulin clearance test.

2-16. **Concentration Tests.** Normal kidneys are able to concentrate urine and efficiently remove waste products from the blood. Increased values for the specific gravity of urine are found in febrile conditions, nephritis, and diabetes mellitus. Decreased values occur in diabetes insipidus and chronic nephritis. Two of the most common concentration tests are the Mosenthal test and the Fishberg test, described in the following paragraphs.

2-17. *Mosenthal test.* The specific gravities of various urine specimens are measured under controlled dietary conditions. On the day of the test, just before breakfast, the patient voids completely and the specimen is discarded. Three normal meals at 0800, 1200, and 1700, each with approximately 1 pint of fluid, are consumed by the patient. No solids or liquids are allowed between meals or after supper. Urine specimens are collected from 1000 through 2000 at 2-hour intervals. Each specimen is collected in a separate container and the time is noted. All specimens voided between 2000 and 0800 the next morning are collected in one container. The volume and specific gravity of each specimen are determined. There should be at least 7 points difference between the lowest and highest specific gravity. The volume of the specimen collected from 2000 hours to 0800 hours is usually at least 600 ml., but less than 800 ml. In kidney disease the volume of the night specimen is usually greater than 600 ml., and the specific gravity varies by only a few points. One specimen should have a specific gravity of at least 1.020 to 1.024. This test is of diagnostic value, except that it is unreliable in heart failure and edema.

2-18. *Fishberg test.* The principle of this test is very similar to the preceeding one. The patient eats a high protein evening meal with fluid intake restricted to not more than 200 ml. No further intake of food or liquid is allowed until the test is completed. The patient is allowed to void and discard urine during the night, but is instructed to collect specimens at 0800, 0900, and 1000 hours. At least one of the specimens should have a specific gravity over 1.024 if the kidneys are normal. In diseased states the specific gravity of all specimens is considerably lower than this.

2-19. **Dilution Tests.** The ability of the kidney to dilute urine is sometimes evaluated by means of dilution tests. Although contraindicated in cases of cardiac or renal edema, it may be of value in conditions such as adrenal insufficiency. Individuals normally excrete 1200 ml. (or slightly more) of urine per day. A patient with adrenal insufficiency (Addison's disease) usually excretes 800 ml. of urine or less in a day. One procedure for conducting a dilution test may be as follows. The patient does not eat or drink after 2000 hours. At 0800 hours he empties his bladder and drinks 1500 ml. of water within 45 minutes. Eight separate specimens are collected at 30-minute intervals thereafter. The specific gravity should be 1.003 or less in at least one specimen and the total volume of urine voided should be over 1200 ml.

2-20. These have been a representative sample of the kidney function tests that are used in the Air Force. While there are others, these should have given you a basic understanding. In addition, we hope that it has motivated you to the constant study and research necessary if you are to keep abreast of the latest techniques in this rapidly changing career field.

CHAPTER 2

Gastric Analysis

A CROWD OF Indians, guides, trappers, and adventurers gathered in the American Fur Company store on June 6, 1822. Someone in the crowd was playing with a gun when it discharged, striking a 19-year-old French Canadian, named Alexis St. Martin, in the stomach. This accident set in motion a series of events and experiments which is the basis of much of what we know about the stomach today. Let's briefly discuss some of these events.

2. An army surgeon, Dr. William Beaumont, was called to treat the unconscious St. Martin. The patient had a hole about the size of a man's fist in his side. After 10 months of conscientious care by Dr. Beaumont, the wound in St. Martin's side was healing, but the opening did not close. As time went on, St. Martin recovered sufficiently to be up and about but the hole remained in his side and stomach. This hole provided Dr. Beaumont with a window through which he could watch what actually happened during digestion.

3. Some of the experiments and observations concerning St. Martin included:

- Giving medications though the hole in St. Martin's stomach.
- Taking temperatures, both inside and outside the stomach.
- Observing the effect of emotional stress on digestion.
- Suspending different foods in St. Martin's stomach and ascertaining the length of time required to digest each.

Dr. Beaumont's experiments and observations were made over a period of several years because St. Martin left and returned many times. The results were finally published in Plattsburg, New York, as *Experiments and Observations on the Gastric Juice and the Physiology of Digestion.*

4. Dr. Beaumont's studies, the first major physiological contribution to research medicine in the United States, was not the first time digestive functions of the stomach had been studied in detail. Lazzaro Spallanzani (1729 to 1799) wrote detailed accounts of digestion based on experiments which he performed. Spallanzani tied strings to pieces of food and then persuaded animals to swallow this food. At intervals thereafter he withdrew the strings and examined the food. It was Spallanzani who correctly noted that certain foods, particularly protein, are broken down in the stomach. Medicine has made much progress since the days of Spallanzani and Dr. Beaumont. In U.S. Air Force hospitals of today, the physician does not peer through a window in his patient's stomach, but he may call upon the laboratory to analyze fluids which have been withdrawn from the patient's stomach. This analysis then helps the physician in diagnosis and treatment of the patient. In this chapter you will study the basic anatomy and physiology of the stomach and laboratory procedures in gastric analysis.

3. Basic Anatomy and Physiology of the Stomach

3-1. Most foods are not naturally in a form which can be absorbed directly into the blood. The stomach serves as a storage area for food until it can be broken down in the intestine. While the food is in the stomach, a mixing action and certain digestive juices reduce the food to a semiliquid state called chyme. The most active components of the digestive juices are hydrochloric acid, lipase, pepsin, and, in infants, rennin.

3-2. **General Features of the Stomach.** The stomach may be described as an expanded part of the digestive tract between the esophagus and the small intestine. The esophagus enters the stomach on the right side just below the fundus. A band of visceral muscle at the bottom of the esophagus constitutes the cardiac sphincter, which remains closed except when a peristaltic wave, or a counterforce such as nausea, causes it to open. Food is forced into the stomach by peristalsis independent of the force of gravity. The narrowing portion of the stomach, below a curving main portion called the body of the stomach, is the pyloric portion. The pylorus is separated from the small intestine by a pyloric sphincter. Peristaltic movements which begin near the middle of the stomach cause food to move into the intestine. Control of the pyloric sphincter is rather complicated and precise. The area between the pylorus and the body of the stomach constitutes the pyloric antrum and canal which are labeled in figure 1. When it is empty, the stomach is arranged in anatomical folds termed rugae. A distended stomach has a capacity of 1 to 1½ quarts in an adult of average size.

3-3. Histologically, the stomach has certain general microscopic features. There are millions of simple tubular glands. Glands in the esophageal area of the stomach differ somewhat from those in other areas of the stomach. Gastric glands are made up of three kinds of cells: the chief cells which secrete enzymes, the parietal cells which produce hydrochloric acid, and the mucous cells. The gastric juice wells up through openings in the mucosa which are referred to as gastric pits. These glands extend to a zone histologically identified as the lamina propria, below which lies the muscularis mucosae.

3-4. The surface of the stomach consists of simple columnar epithelium, which extends down to line the pits. This epithelium protects the surface of the stomach. Normal and viable stomach tissue is not affected by the digestive juices present in the stomach, although immediately after death, cells of the stomach undergo autolysis. As might be expected, ulceration of the mucous membrane sometimes occurs. Contrary to what you might expect, wounds of the stomach heal perfectly well even in the absence of the protective columnar epithelium. Psychogenic factors may complicate healing processes.

3-5. **Gastric Digestion.** The primary functions of the stomach are (1) storage of food, (2) digestion, and (3) bactericidal effect. All are important, but not vital, since complete removal of the stomach (total gastrectomy) can be tolerated. Food which passes through the cardiac

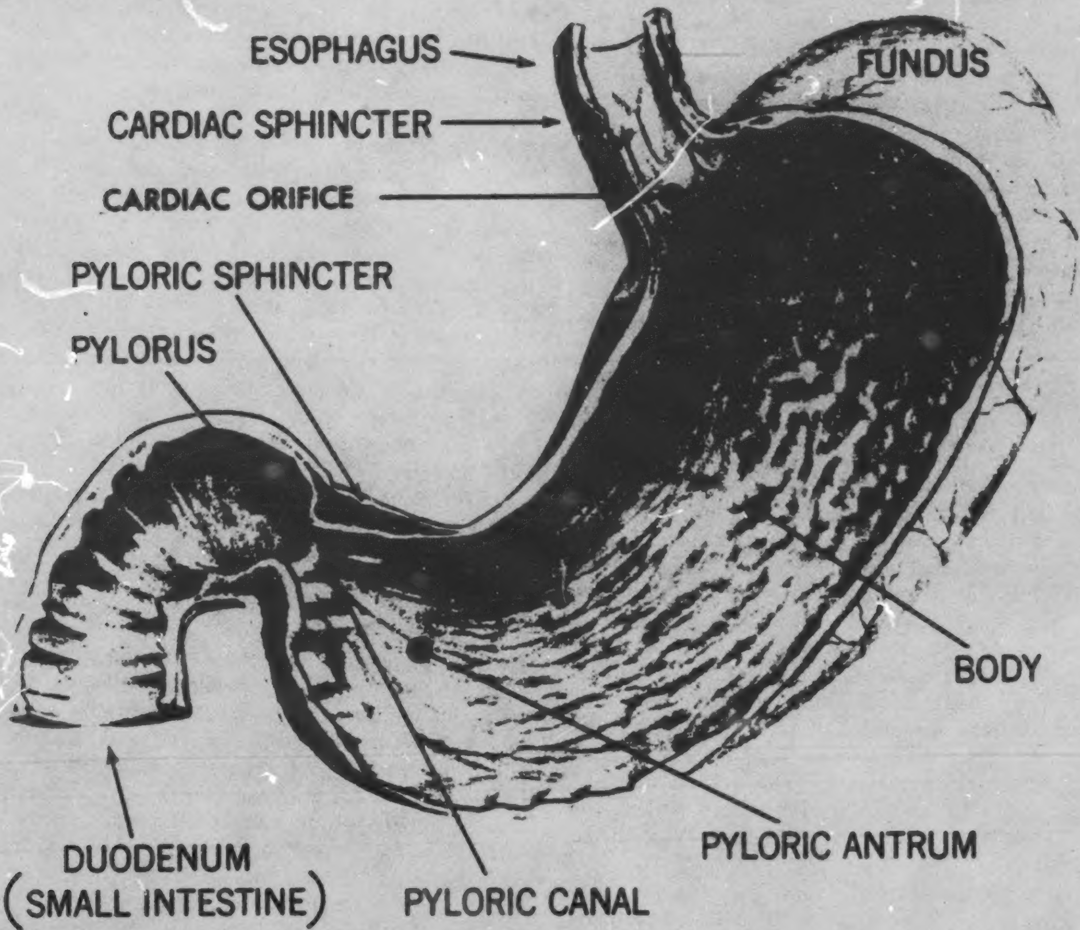


Figure 1. The stomach.

orifice of the stomach accumulates in the lower curved portion. Further accumulation of food causes some stretching of the stomach walls. Peristalsis of the stomach is regulated according to the amount of food present. Secretion of gastric juices is caused by nerve impulses which are conducted from the brain through the vagi nerves to the glands of the stomach. These impulses are the result of sensory or psychic stimuli including the sight or smell of food. Stretching the stomach walls, as well as the chemical nature of certain foods, also influences gastric secretion. Alcohol and histamine are both used in the hospital to stimulate gastric secretion.

3-6. Gastric juice consists of 98 percent water; the rest includes pepsin, hydrochloric acid, inorganic salts, mucin, and the so-called intrinsic factor. This is a factor present in gastric juice which is essential for the prevention of anemia. Its exact nature is obscure, but it is thought to be a mucoprotein secreted in the fundus and cardiac portions of the stomach. Gastric juice has a pH of 0.9 to 1.5 and is about 0.15*N*. The amount of gastric juice found in the fasting stomach is usually about 50 ml., with approximately 2500 ml. secreted per day by an adult. Patients with a duodenal ulcer secrete twice that amount. The actual process of HCl formation in a parietal cell is essentially that of the chloride shift described in an earlier chapter. Carbonic acid, H_2CO_3, is the source of the hydrogen that combines with chloride ions which diffuse into the cell from the surrounding plasma.

3-7. Besides the constituents of gastric juice mentioned, certain other substances are present. They include pepsin, rennin, and lipase. Together with the action of the acid, pepsin breaks proteins into proteoses and peptones. Rennin, which is of little consequence to adults, converts milk protein (casein) to paracasein, and lipase acts on fats to produce fatty acids and alcohols. The lipolytic action of gastric juice is not considered very important, because of the greater activity of pancreatic lipase in the intestine. Gastric lipase is responsible for the release of butyric fat from butter fat, a product which causes the characteristic disagreeable odor of gastric contents. Unlike the intestine, relatively little absorption takes place in the stomach. Water and alcohol are examples of substances which can be absorbed directly into the blood from the stomach.

3-8. **Gastric Acidity.** Total gastric acidity depends upon the presence of both free and combined acid. Combined acid includes HCl combined with protein, acid phosphates, and organic acids. Gastric acidity is expressed in degrees. A *degree* is the amount of acid in 100 ml. of gastric juice which will be neutralized by 1 ml. of 0.1*N* NaOH. One degree is numerically equal to 1 mEq/L. because the milliliter of 0.1*N* NaOH required to neutralize 100 ml. of gastric juice is equal to the ml. of 1.0*N* to neutralize 1,000 ml. This entity meets the definition of an equivalent weight in grams, which is also numerically equal to a milliequivalent weight in milligrams.

3-9. There is a correlation between the gastric secretory activity and certain clinical conditions. Hyperacidity commonly occurs in cases of duodenal ulcer, whereas hypoacidity is characteristic of gastric carcinoma. The absence of hydrochloric acid (achlorhydria) is indicative of pernicious anemia. The degree of acidity varies according to whether or not the patient is fasting. A normal fasting specimen is as follows:

Free acidity: 5° to 20°
Total acidity: 15° to 45°

Very often the physician is interested in knowing only whether free acid is present. Analyses are also performed following test meals. Many of the test meals used in former years are no longer used today. Some of these, such as the Ewald meal, consisted of toast and tea. The most common means of stimulation today are through the use of alcohol, caffeine, and histamine. Insulin also stimulates gastric secretion with accompanying hypoglycemia. Stimulating gastric secretion is almost exclusively accomplished by the medical and nursing staff of Air Force facilities, as is the use of a gastric tube for withdrawal of stomach contents. It is not necessary, therefore, that you, the laboratory technician, be familiar with the details of these procedures. You should be aware of the nature of gastric stimulants from the viewpoint of physiological chemistry, however.

3-10. Normal patients will show maximum production of gastric juice about 30 minutes after ingesting 500 mg. caffeine sodium benzoate. The level then drops sharply. Histamine is injected subcutaneously to the extent of 0.01 mg./kg. body weight. The usual dose is 0.3 ml. of a 1/1000 histamine phosphate solution. Specimens are withdrawn at 15-minute intervals for up to 90 minutes. The normal response is similar to that observed with caffeine and alcohol. Ulcer patients do not show a marked drop in HCl, whereas normal subjects show a definite decline in HCl production at 60 to 80 minutes after stimulation. Although interpretation of the response is not a laboratory problem, you should appreciate that there are three main categories of abnormal response to gastric stimulation—achlorhydria, hyperacidity, and hypoacidity.

3-11. Achlorhydria is characterized by the absence of free HCl in all specimens. This is usually found in patients with diabetes mellitus, adrenal insufficiency, pulmonary tuberculosis, arteriosclerosis, and hyperthyroidism. Achlorhydria is occasionally confused with false achlorhydria, which is the absence of free HCl due to neutralizing substances in the specimen. The two conditions may be distinguished in the laboratory by analyzing the gastric fluid for chloride.

3-12. Hyperacidity is the response indicated by high acid values, continuing during the 60- to 90-minute interval which is the digestive phase. The increase may or may not level off. Various types of curves are encountered which may be indicative of duodenal ulcer, gastric neurosis, cholecystitis, hyperirritability, gastric ulcer, and appendicitis. In some cases very little clinical significance can be attached to a sharp rise in gastric acidity.

3-13. Hypoacidity is a condition in which the response is less than normal. This is observed in carcinoma of the stomach, gastritis, neuroses, colitis, anemia, and gastric ulcer, as well as in a few other conditions. A low level of gastric secretion is also observed in about 20 percent of the normal population and in a significantly greater proportion of pregnant women.

4. Laboratory Procedures in Gastric Analysis

4-1. Of primary concern to the clinical laboratory is the acidity of gastric fluid because of its relationship to clinical conditions. Acidity is due to both free and combined acid. For example, in conditions such as carcinoma of the stomach there may be little or no acid. Hyperacidity, on the other hand, may indicate a gastric ulcer. Acidity is determined by a titration procedure described in the following paragraphs. With this titration you will be able to differentiate total from free HCl. The presence or absence of free HCl can sometimes be determined by a tubeless test. In a tubeless analysis we measure in the urine the amount of dye displaced from an ion exchange resin by HCl as the resin passes through the patient's stomach. This technique is discussed below in paragraph 4-4.

4-2. **Physical Characteristics of Gastric Fluid.** The following characteristics should be noted as you assay gastric fluids. Any deviation from the normal should be reported.

a. Color. A normal-appearing, fasting gastric specimen is opaque to clear, with a slight grey to pale yellow color.

b. Presence of mucous. Gastric fluid may be difficult to pipette because of its viscous nature. A 10-ml., wide bore tip, serological pipette equipped with a bulb works well in transferring the specimen. A specimen may also be carefully measured in a 10-ml. graduated cylinder. Preliminary filtration through gauze is helpful. Abnormal amounts of mucous may indicate gastritis, with inflammation of the mucous tissue. Excessive mucous may cause falsely decreased acid values.

c. Volume. The volume of the gastric specimen should be measured.

d. Odor. This is usually less important than other features, though an unusual odor may be of interest. For example, a fecal odor is typical of intestinal obstruction.

e. Particulate matter. Food particles are not normally present in a fasting specimen unless there is a problem of stasis.

f. Presence of blood. Blood which has accumulated in the stomach is brown, usually described as appearing like coffee grounds. Bright red blood due to fresh bleeding is less common unless it results from trauma or injury. The presence or absence of blood is extremely important and should be tested for by the guaiac or benzidine test if suspected.

4-3. **Titration of Acid.** The principle of gastric titration is as follows: The concentration of free hydrochloric acid is measured by titration with standard alkali to the Topfer's reagent (known chemically as dimethylaminoazobenze—methyl yellow) endpoint (pH 3.3), and the total acidity by titration to the phenolphthalein endpoint (pH 8.5). Because two separate titrations are performed on the same specimen, this procedure becomes confusing to many technicians. A simple version of the titration procedure is outlined below. Let's first look at the step-by-step method of performing the titration.

Step 1: Label a 50-ml. Erlenmeyer flask for each specimen and place 5 ml. of specimen in each flask.

Step 2: Add 3 drops of Topfer's reagent to each flask, mix by swirling, and note the color change. Yellow indicates *no* free acid is present. Red indicates the presence of free acid.

Step 3: If there is *no* free acid, record this and proceed to Step 5.

Step 4: Free Acidity. If free acid is present, titrate to the salmon-pink endpoint of Topfer's reagent with 0.1*N* sodium hydroxide. Record the amount of 0.1*N* sodium hydroxide used. This figure is used to calculate free acidity.

Step 5: Add 3 drops of phenolphthalein indicator solution.

Step 6: Combined Acidity. Titrate to the pink-red endpoint of phenolphthalein. Record the amount of 0.1*N* sodium hydroxide used as the amount required to titrate combined hydrochloric acid.

After making the step-by-step titrations you are now ready to make the calculations as follows:

Calculation for free HCl

$$\frac{\text{ml. of NaOH from step 4}}{\text{ml. of gastric specimen}} \times 100 = \text{degrees free from HCl}$$

Calculation for combined HCl

$$\frac{\text{ml. of NaOH from step 6}}{\text{ml. of gastric specimen}} \times 100 = \text{degrees combined HCl}$$

Total = combined + free.

NOTE: Since total = free + combined, combined = total − free and represents that portion of the gastric HCl which is combined with organic substances.

4-4. **Tubeless Gastric Analysis.** An ion exchange resin is available commercially (Diagnex-Blue—Squibb) which makes use of a dye, azure A, as an indicator. The patient is given a packet of the resin granules and instructed to swallow them without chewing, as outlined on each package. The amount of dye which appears in the urine depends upon displacement from the resin by HCl in the stomach. A color chart is used to estimate the amount of dye in the urine, which is an index of the amount of HCl in the stomach. This test is considered valuable as a screening test only. It is preferred to passing a gastric tube only because it is simple and does not discomfort the patient. Its reliability, however, is not beyond question.

4-5. Within the past few years there have been attempts to take a new approach to the subject of gastric analysis in keeping with up-to-date methods and equipment. Some investigators have proposed abandoning the terms *total* and *free* acid and replacing them by the more meaningful terms *hydrogen ion concentration* $[H^+]$, *undissociated hydrogen ion concentration* $[C_H]$ and *titratable acidity*.[1] A change in the concept of gastric analysis as proposed is a good example of applying new ideas and greater sophistication to basic laboratory procedures.

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[1] Moore, E. W.; Scarlata R. W. "Gastroenterology," 49: 178–188, Aug. 65, "The Determination of Gastric Acidity by the Glass Electrode."

CHAPTER 3

Special Chemistry Tests

SPECIAL CHEMISTRY TESTS are "special" only because these tests are usually performed only in large clinical laboratories. This does not mean they are unnecessary at small medical facilities, but special chemical analyses usually require unique, costly equipment, and technicians trained in the special techniques. Because of the cost and complexities of these tests, it is important for all technicians at reference and referring laboratories to be aware of general technical considerations involved with each "special" test.

5. Referral Chemistries

5-1. In this section we will consider various referral-type analyses. The discussion of electrophoresis develops from variations in technique. Chromatography is reviewed in principle and in its general application. The physiology and quantitation of serum iron is developed in the light of its clinical importance, while recent dramatic innovations in protein-bound iodine (PBI) analysis are described in comparison to standard manual methods. Hormones are briefly defined relative to their importance in clinical medicine. Qualitative urinary calculi analyses are covered briefly.

5-2. These are the more common special chemistry tests. You will need to know this information to understand your responsibilities in collection, preservation, and shipment of specimens. You may also require the technical knowledge of special chemistry procedures to actually perform some of these tests in a large reference laboratory.

5-3. **Electrophoresis.** The migration of charged particles in an electrolyte solution resulting from an electric current flow is known as electrophoresis. Colloidal particles, such as protein, although invisible to the unaided eye, are actually in suspension rather than in true solution. Some of the particles (fractions) which may be separated by electrophoresis because of their different mobilities include proteins, lipoproteins, hemoglobins, amino acids, and many other substances of medical and biological importance. The widespread application of electrophoresis is quite recent. However, the concept of electrophoresis is not new.

5-4. In 1861, a German investigator, Quincke, demonstrated a relationship between the speed of particle migration and the electric potential gradient, as well as the relationship between speed of migration and pH of the suspending medium. Egg albumin was one of the proteins first investigated in detail. The activity of albumin in an electrical field was studied by W. B. Hardy in the latter part of the 19th century. Writing in the *Journal of Physiology* in 1899, Hardy reported that protein particles have ". . . this very interesting property that their electrical characters are conferred upon them by the nature of the reaction, acid or alkaline . . ." In other words, particles, such as proteins, suspended in an aqueous medium will have either a positive or a negative charge depending upon the pH of the solvent and the nature (size, charge, etc.) of the particles. The pH at which a protein is electrically neutral is the point at which it will not migrate in an electrophoretic system. This particular pH, characteristic for each protein, is referred to as its *isoelectric point*. The major protein fractions of human serum are in the anionic form at an alkaline pH and, hence, will migrate to the anode. They are usually separated at a pH of 8.6.

5-5. A major problem in the development of electrophoresis has been quantitating each fraction which can be distinguished. Keep in mind that both *rate* and *direction* of movement in a field depend upon the nature of the particle itself as well as the pH of the solution. Albumin, for example, can be separated from globulin because it migrates at a faster rate because of the size and charge of the albumin molecule. Quantitating the albumin is quite another matter, however. The first practical means of quantitatively separating proteins was described in 1937 by Nobel Prize Winner Arne Tiselius of Sweden. The Tiselius apparatus is used today in research and special studies, but it is not particularly useful in a hospital laboratory. This type of instrument represents *moving boundary electrophoresis.* The use of supporting media to prevent mixing of the fractions by convection is more recent and more practical for clinical use.

5-6. *Serum.* The use of supporting media such as starch-gel, paper, agar-gel, and cellulose acetate through which the proteins migrate is referred to as *zone electrophoresis.* Most familiar to the clinical laboratory is the equipment pictured in figure 2 which uses paper strips. The serum to be separated is placed in the middle of an electrolyte-buffer-saturated paper strip. Electrodes are applied to each end of the strip in a tank of buffer solution. Sixteen to 24 hours are required for adequate separation of all serum-protein fractions. After the current is disconnected, strips are dried and developed with a dye (e.g. bromphenol blue) to stain bands of proteins. Reading from anode to cathode on the strip, serum fractions are albumin, alpha 1, alpha 2, beta, and gamma (α_1, α_2, β, and γ) globulin. The relative amount of each component may be charted with a scanning device which measures the density of each band by a photocell as indicated in figures 3 and 4. A curve is charted with peaks to represent each fraction which may then be expressed quantitatively as a percent of the total. Percent is determined from integration units marked on the x-axis by the scanning instrument. To determine the actual amount of component in grams percent or milligrams percent, the percent figure is multiplied by a total concentration value obtained by some conventional means of analysis, such as the biuret method for proteins. If a scanning instrument is not available, bands may be eluted from segments of the paper strip with a suitable solvent and read in a cuvette with an ordinary spectrophotometer. Normal values for protein fractions in serum are given in table 4. Serum specimens are stable for at least 3 days at room temperature and for at least a month if refrigerated.

5-7. Electrophoretic studies are clinically useful in patients with liver diseases, myeloma, chronic infections, and sickle-cell anemia as well as in certain other conditions. Compare the tracing of serum from multiple myeloma in figure 3 with the normal serum protein electrophoresis in figure 4. A relatively new method which shows promise uses cellulose acetate strips as a support medium. Speed, improved resolution, and microsample size are distinct advantages with this supporting medium. Resolution of 0.25 microliters of serum is completed in ½ hour as opposed to 16 hours on paper. The terms microliter (μl) and lambda (λ) are synonymous and equal to 1/1000 ml. In addition eight different specimens can be placed on one strip of cellulose acetate, whereas with paper each specimen requires a single paper strip for resolution.

5-8. *Hemoglobin.* Hemoglobin fractions may be separated with paper electrophoresis; however, it is not as sensitive as other methods. For example, it will not detect less than 15 percent of hemoglobin S (Hb-S) in a mixture of normal adult hemoglobin (Hb-A) and Hb-S, nor does it allow distinction of minor components such as Hb-A$_2$. Variations in mobility of different electrophoretic fractions may result from slight changes in buffer pH or ionic strength and fluctuations in temperature or electric current. These differences from one electrophoretic run to another make the use of known hemoglobin fractions an essential requirement for each hemoglobin electrophoresis. These standard hemoglobin fractions may be procured commercially or prepared locally from known patient sources. The relative mobilities of human hemoglobins after paper electrophoresis starting with the least migration are:

C < E < S&D < F < G < A < I < J < H

The common hemoglobin fractions Hb-A and Hb-F (fetal) are not distinguishable after migration, so an alkali denaturation technique should be used on all specimens to determine the percent of Hb-F which is resistant to this denaturation. Normal adults and children over 4 years should have less than 2 percent Hb-F. On any specimens migrating as Hb-S, a ferrohemoglobin solubility may be employed to rule out the possibility of the fraction being the rare Hb-D which migrates at the same speed as Hb-S. Hb-S is relatively insoluble, while Hb-D and most other hemoglobins are quite soluble.

5-9. Other methods are available which render more sensitive resolutions of hemoglobin variants. One method uses starch block as the supporting medium. This has a distinct advantage in the separation of Hb-A and A$_2$ fractions necessary for the diagnosis of thalassemia minor. It has the disadvantage of being very difficult to use; and, in addition, permanent records can be obtained only with photographs.

5-10. *Cellulose acetate.* Cellulose acetate is rapidly becoming the medium of choice for both routine serum and hemoglobin electrophoresis. The advantages in serum protein fractionation have already been mentioned. These same factors—resolution, speed, and micro-sample size—are also desirable in hemoglobin electrophoresis. Cellulose acetate electrophoresis will resolve (separate) Hb-A$_2$ from Hb-S and Hb-A from Hb-F in less than 2 hours of electrophoresis. Hb-F concentrations of less than 2 percent have been quantitated on cellulose acetate strips. In

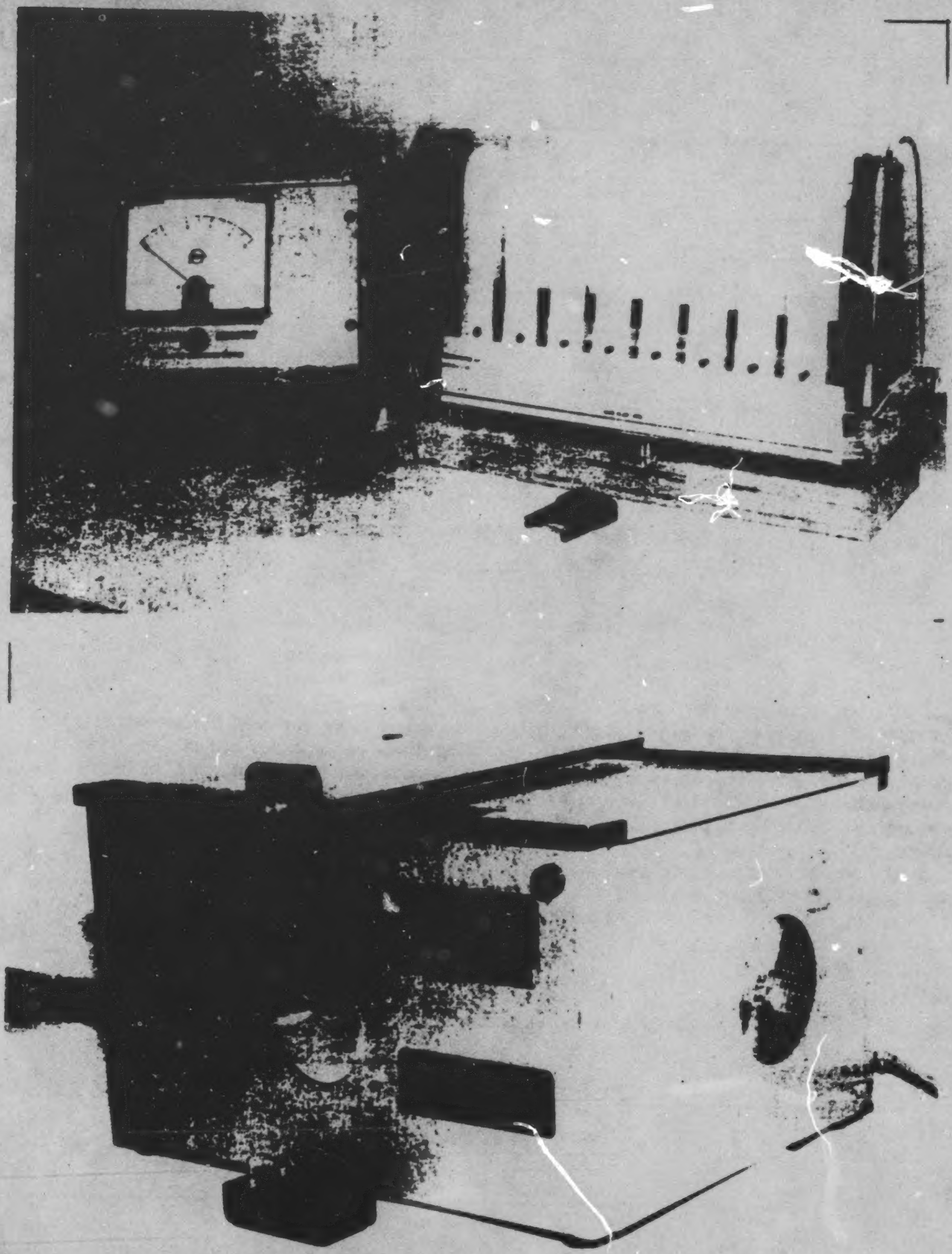


Figure 2. Equipment to perform *zone electrophoresis* using paper strips.

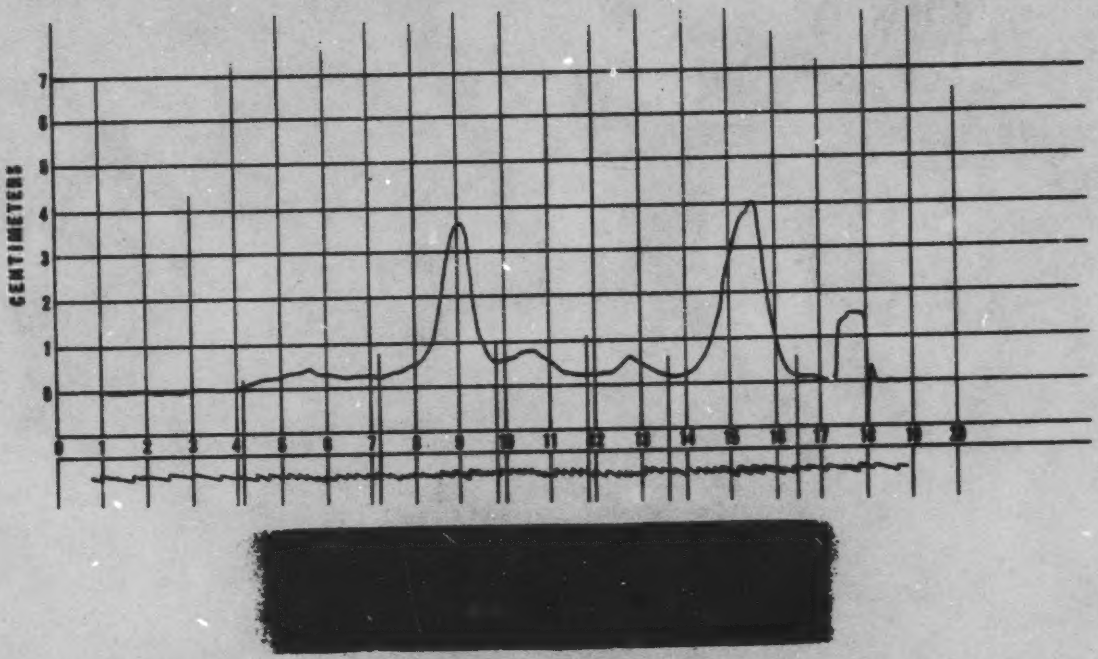


Figure 3. Tracing of serum from multiple myeloma.

addition, the strip may be cleared to transparency for more accurate quantitation of fractions. These advantages and the marketing of modification kits for existing paper electrophoresis equipment have enhanced the use of this medium for routine electrophoresis in the clinical laboratory.

5-11. Various techniques are used in preparing blood specimens for separation of hemoglobin variants. In one method of preparing a hemolyzate, red cells are washed with saline after which a volume of water equal to the original volume and 0.4 volumes of toluene are added. Centrifugation separates cellular debris resulting in a clear hemolyzate containing the hemoglobin. The use of a buffer is sometimes advocated to avoid possible loss of certain abnormal hemoglobin fractions. Another method of preparing a hemolyzate is by freezing and thawing, though some investigators find this produces some protein denaturation. The maintenance of known abnormal control hemolyzates is desirable. Clear

TABLE 4

NORMAL VALUES FOR SERUM PROTEIN FRACTIONS BY ELECTROPHORESIS PAPER

Component	*Percent Total Protein*	*g./percent*
Total Protein		6.2 to 8.5
Albumin	52 to 68	3.5 to 5.5
Globulins		
Alpha 1	2.4 to 5.3	0.2 to 0.4
Alpha 2	6.6 to 13.5	0.5 to 0.9
Beta	8.5 to 14.5	0.6 to 1.1
Gamma	10.7 to 21.0	0.7 to 1.7

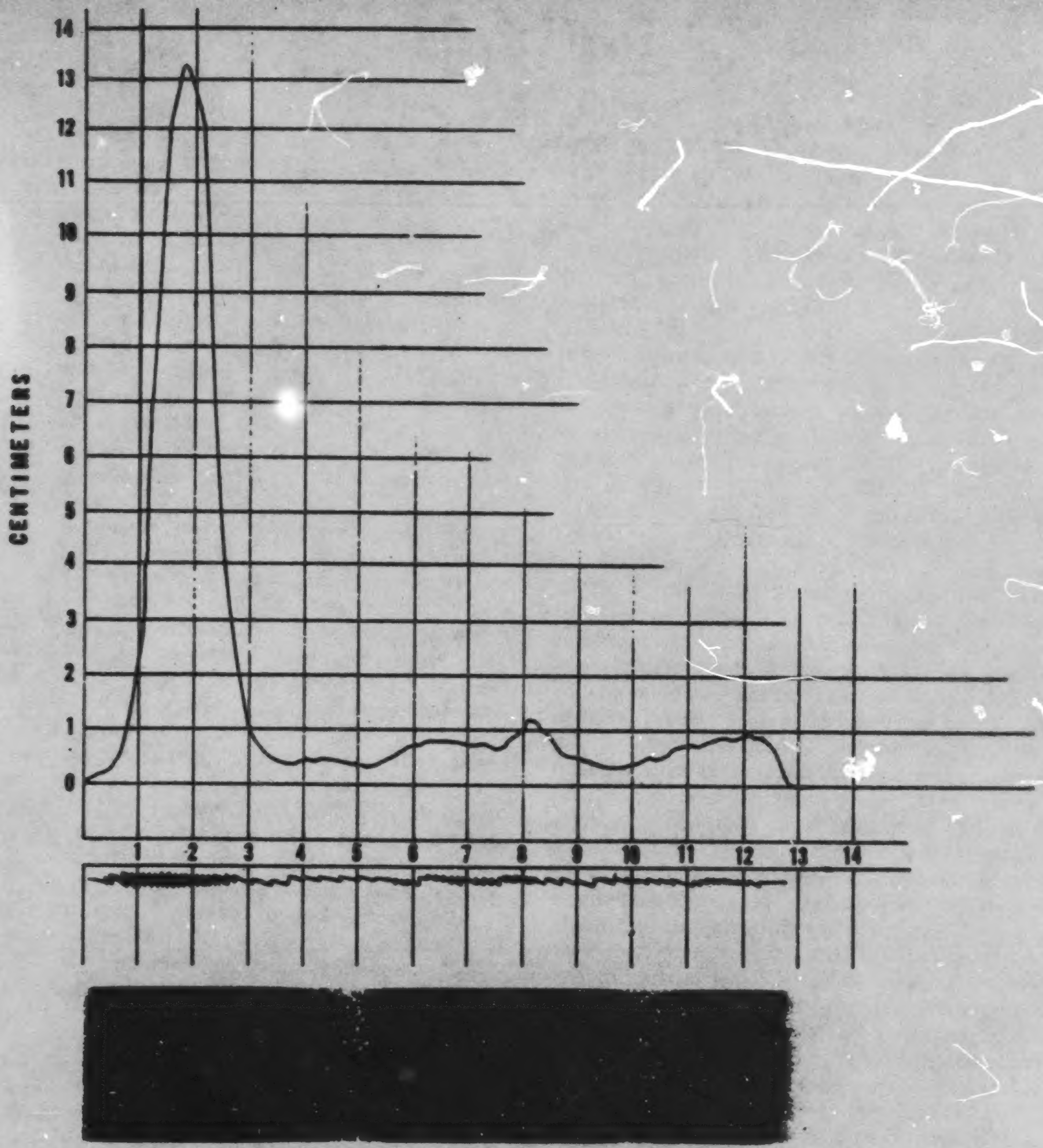


Figure 4. Tracing with normal serum protein electrophoresis.

hemolyzates may be stored for months in sealed capillary tubes at −10°C. Capillary tubes provide sufficient small quantities and reduce the risk of excessive denaturation from repeated thawing and freezing of single large specimens. Denaturation of control samples will be evidenced by increased trailing (spreading) of the protein bands.

5-12. **Chromatography.** Different solutes move through absorption media at different rates and, hence, can be separated from each other. This principle is the basis of chromatography. Although many investigators have contributed to the development of chromatography, principles applied today are essentially those developed by two early investigators, David Talbot Day (1859–1925) and Mikhail Tswett (1872–1919). Dr. Day was a geologist who, while working for the U.S. Government, observed the various colors of mineral samples. He attributed the color layers to "fractional filtration." He demonstrated that when crude oil was passed through finely pulverized earth, the first fraction was different from the second fraction; the second was different from the third, and so on. At approximately the same time Day carried on his experiments in the United States, an Italian-born Russian physical chemist, Professor Mikhail Tswett, was working with pigments in leaves. He observed that when a petroleum ether extract was filtered through a column of adsorbent material, the pigments which were dissolved in the solution were resolved according to their adsorption sequence as colored zones throughout the medium. Although separation today often includes colorless substances, the name *chromatography* is retained.

5-13. There are many variations of chromatographic separation in use, both on a limited scale and on an industrial scale. It is applied to gases as well as to liquids. Different types of adsorbing materials include liquids and solids, cationic and anionic resins, polar and nonpolar liquids, paper, and a variety of other substances. Through diverse modification, chromatography has been applied to all kinds of gaseous, volatile, and soluble materials. Gas chromatography, column chromatography, and paper chromatography are three of the most common applications of separation by differential migration. Gas chromatography is divided into two categories, gas partition and gas adsorption. In addition to the above described methods of chromatography, a direct electric potential can be applied to promote separation of components in a stabilized electrolytic solution. (This is known as electrochromatography, and should not be confused with electrophoresis.) Gas chromatography and paper chromatography have been used to assay various substances in the clinical laboratory, including blood gases, bile pigments, alcohol, sugars, amino acids, drugs, and many other chemicals.

5-14. **Serum Iron and Iron-Binding Capacity.** The iron reserves of the body are stored as ferritin in the liver, spleen, bone marrow, and mucosal cells of the intestine. Absorption of iron is limited by the iron-binding capacity of the intestinal mucosa. Intestinal absorption, erythropoiesis, hemoglobin catabolism, and siderophilin metabolism are all factors which influence the serum (or plasma) iron level.

5-15. A clinically significant iron concentration is present in plasma or serum in excess of that normally present as hemoglobin. The normal values reported vary with the quantitative method, but are in the range of 65 to 175 μg percent. Earlier methods reported lower values in females but more recent studies found no sex difference. This nonhemoglobin serum iron is known as transport iron, acid-soluble iron, loosely bound iron, or protein-bound iron. Nonhemoglobin iron is not the total iron reserves but rather iron in transit from one part of the body to another. Siderophilin (transferrin) is a β globulin which loosely binds nonhemoglobin iron during its transport. Only about one-third of the available siderophilin is normally bound to iron. This is the serum iron. The remaining unsaturated siderophilin constitutes 60 to 70 percent of the available siderophilin. This unsaturated siderophilin is the unsaturated iron-binding capacity (UIBC) or latent iron-binding capacity (LIBC). Serum iron plus the UIBC is the total iron-binding capacity (TIBC) of serum. Serum iron expressed as a percentage of total iron-binding capacity is the percent saturation.

$$\frac{\text{Serum iron}}{\text{TIBC}} \times 100 = \text{percent saturation}$$

5-16. Quantitation of serum iron involves first splitting off the iron from its carrier protein. This is usually done with hydrochloric acid (AFM 160–49, *Laboratory Procedures in Clinical Chemistry*). Then the proteins are precipitated with trichloracetic acid, and the supernate is reacted with nitric acid and potassium thiocyanate for color development. Another method uses hot trichloracetic acid which simultaneously releases the iron and precipitates protein. This method continues with reduction of iron using hydrazine sulfate and color development with sulfonated bathophenanthroline.

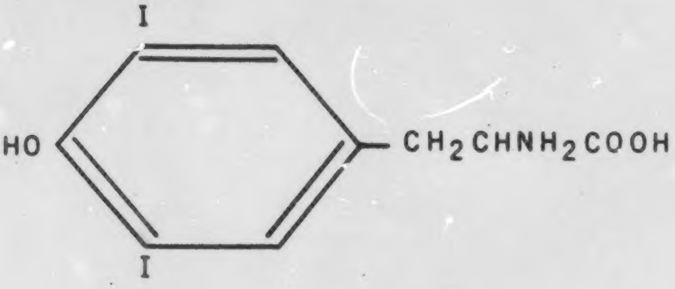
5-17. **Protein-Bound Iodine.** Thyroxine was first isolated in 1915. Since 1939 when the relationship of iodine to protein circulating as thyroxine was presented, there has been an increasing demand for the determination of thyroxine in the clinical laboratory. Iodine exists in serum as thyroxine (80 to 90 percent), inorganic iodine (10 percent), diiodotyrosine, triiodothyronine, and diiodothyronine. Thyroxine is the compound of interest in the evaluation of metabolic processes involving the thyroid gland. Diiodotyrosines are assumed to be precursors of the thyroxine molecule. Note the similarities of their structural formulas in figure 5. Thyroxine circulates in loose association with a protein in the blood plasma, thus, the term protein-bound iodine (PBI).

5-18. Early efforts to assay iodine as a measure of thyroxine were inadequate because of a lack of suitable chemical reagents. The methods developed over the years have been many and varied. All chemical analyses for PBI do have certain aspects in common. They are:

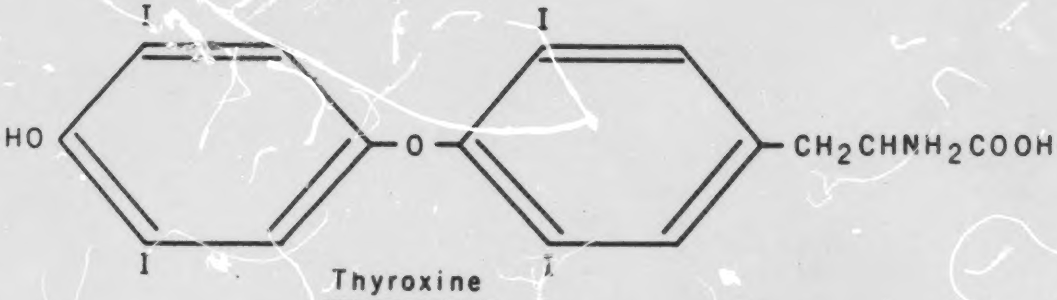
a. Separation of organic iodine or thyroxine from other iodine.

b. Digestion of organic iodine molecules to release inorganic iodine.

c. Quantitation of inorganic iodine.



3,5-Diiodotyrosine



Thyroxine

Figure 5. Structural formulas of Diiodotyrosine and Thyroxine.

5-19. Butanol extraction was an attempt to separate the organic iodine from other iodine compounds in serum. Inorganic iodine and the diiodotyrosines may be separated from thyroxine and the other normal organic iodine compounds with n-butanol. This is the butanol-extractable iodine or BEI. Organic contamination, such as radiographic dyes used in X-ray, are not separated from thyroxine in butanol extraction. More recently ion exchange resins have been used successfully to remove normal inorganic iodine compounds. Exchange resins do not eliminate organic iodine contamination. Precipitation of the protein-containing iodine (PBI) with various methods involves many techniques and modifications presented over the years. The Somogyi precipitation, consisting of zinc sulfate and sodium hydroxide, is one method used extensively at present. Trichloroacetic acid (TCA), tungstic acid, and perchloric acid as well as the application of heat with certain acids have been used. Radioactive I^{131} studies on washed precipitates revealed 15 percent residual I^{131} in TCA precipitates, and 1 percent residual in Somogyi precipitates. Ion exchange resins, however, remove over 99 percent of normal inorganic iodine levels from serum and require considerably less effort.

5-20. After organic iodine is separated, it must be treated to release inorganic iodine for quantitation. Generally, procedures for PBI employ acid digestion (wet digestion) or incineration (dry ashing) for this purpose. The original wet digestion methods required distillation to obtain inorganic iodine for quantitation. Modifications using permanganate, perchloric acid, and chromic acid have been introduced for direct quantitation without distillation. The normal values of some direct procedures have been reported to be higher than dry ashing techniques. Residual inorganic iodine in certain protein precipitates could account for the increased values. Normal values are generally 4 to 8 µg. I/100 ml., but this varies somewhat depending upon the technique and geographic area.

5-21. Alkaline incineration, or dry ashing is by far the manual method most extensively used for PBI determinations. Dry ash techniques in general follow Barker's modification of an earlier report. Recent modifications use ion exchange resins initially to prepare the serum. A Somogyi precipitated protein-bound iodine is washed and sodium carbonate is added to reduce loss of iodine during incineration. The mixture is dried and incinerated at 600 ± 25° C. for 2 to 3 hours. The residue is dissolved in acid and quantitated, using the ceric-arsenious acid reaction. Thyroxine recovery experiments have indicated recovery values of 93 ± 3.7 percent with the Barker dry ash procedure.

5-22. So far in PBI analysis we have considered methods for separating the organic iodine and digestion of the organic residue to release inorganic iodine. The final general consideration is quantitation of released iodine. All methods in use at present employ the ceric-arsenious acid reaction for colorimetric determination of iodine from PBI. This reaction involves reduction of yellow-colored ceric ions to colorless cerous ions by arsenious acid through the catalytic effect of iodide ions. There is a linear relationship between reaction time and iodide concentration. The *rate* of reaction or *change* in optical density rather than a final stable optical density determines the concentration thus obtained. Timing intervals between O.D. readings becomes an important factor in this colorimetry. For this reason, the number of assays determined at one time depends upon the manual dexterity of the technician.

5-23. It is impossible to discuss PBIs without mentioning the ever-present problem of contamination. Probably no other test is more prone to contamination than the PBI. Contamination may come from the patient. This exogenous iodine may result from radiopaque dyes used in cholecystography, urography or myelography; iodinated amoebicides; vaginal suppositories containing diiodohydroxyquin and iodothiouracil; tinctures of iodine, merthiolate, mercurochrome; or the use of "all weather" suntan lotions containing iodine. Inorganic iodine contamination may also result from technicians who collect specimens after working with Gram's stain, or Lugol's solution. Water used in PBI analysis is often a source of contamination. It must be glass redistilled and deionized before use. All glassware must be scrupulously cleaned in chromic acid and thoroughly rinsed.

5-24. Problems in PBI analysis often defy explanation. Manual methods are tedious, time-consuming, and often frustrating. Proficiency depends upon so many controlled and uncontrollable circumstances. A janitor may innocently mop a floor with required disinfectant resulting in enormous and long-lasting residual iodine contamination. Mercury is often spilled and not retrieved, leaving its inhibitory effect. Nessler's reagent may be used and thereby contaminate either the specimens, glassware, or reagents.

5-25. One of the most exciting advances in automation for the clinical chemist was the introduction in 1964 of a completely automated system for PBI. This was accomplished with an autoanalyzer. A partially automated system was developed in 1963. The original semiautomation resolved time-reaction problems by introducing a constant-moving stream of the ceric-arsenite reactants. Reactants were automatically added to the stream in constant proportion, mixed, heat-developed, and read photometrically at constant intervals. The serum was pretreated with ion exchange resins to remove inorganic iodine. Complete automation was subsequently achieved with an automatic protein digestor module. A mixture of perchloric, sulfuric, and nitric acid forms the digestion reagent. The acid reagent is fed automatically into a spiral, grooved glass helix rotating on electric heating rods. Heat and acid effectively digest the serum protein, releasing iodine. Then, the colorimetry of the former partial automation is applied automatically to the digest as it is aspirated from the protein digestor module. A screening run at 120 specimens/hour identifies contaminated specimens. These are removed and the uncontaminated specimens are run at 20/hour for quantitation. Contaminated specimens cannot be run with other specimens because one such specimen will contaminate the system for the following 4 or 5 specimens.

6. Hormones

6-1. A hormone is a chemical substance produced by an endocrine gland, i.e., a gland of internal secretion. One exception to this definition is the placenta which produces hormones, but which is not strictly speaking an endocrine gland. You are undoubtedly aware that it is placental gonadotropin which you measure in pregnancy testing. The purpose of a hormone is to control the functional activity of another part of the body. For example, certain hormones secreted by the pituitary gland control thyroid activity. The effect of hormones is measured indirectly in the laboratory in practically every test that is performed. For example, a blood glucose result reflects the activity of the adrenal and pituitary glands. This was explained in a previous chapter concerning glucose. The purpose of the present discussion, however, is to point out that a more direct approach is possible by studying and evaluating endocrine functions. Specifically, each hormone or group of hormones can be directly assayed in the laboratory. Two major classifications discussed here are steroid hormones and pituitary hormones.

6-2. **Steroid Hormones.** The group of hormones known as steroid hormones are characterized by the ring configuration shown in figure 6. This particular 17-carbon atom structure is known as the cyclopentenophenanthrene ring. (Note the similarity to cholesterol described earlier in this course. In fact, all adrenal steroid hormones are derived from cholesterol.) The assay of steroid hormones is of considerable medical interest in the study of certain functional disorders. Unfortunately some of these analyses are beyond the capability of the average medical laboratory. A clear understanding of these tests and their limitations is essential for proper interpretation of your technical responsibilities. The following is a brief review of *steroid hormones* and the methods of analysis available to clinical laboratories.

6-3. *Estrogens.* Included are estradiol, estrone, equilenin, and estriol. An assay of the individual estrogens is seldom of clinical importance; hence, total estrogens only are considered. Estrogens are formed in the ovaries, the adrenal cortex, and the placenta. They are secreted mainly in the urine as glucuronidates and sulfates. Estrogens are usually assayed by vaginal cytologic examination which is an estimation of the estrogen activity. Bioassay, colorimetric, and fluorometric methods are either nonspecific or too involved to be performed in a clinical laboratory. Decreased estrogen levels lead to sexual immaturity in the female. Increased values are most commonly associated with tumors of the ovaries.

6-4. *Progesterone.* Formed in the corpus luteum of the ovary, its concentration closely parallels the development, activity, and regression of the corpus luteum. It is also formed in the adrenal cortex as an intermediate compound in the biosynthesis of adrenal corticoids. The reduction product, pregnanediol (progesterone is always excreted as the glucuronide of pregnanediol in the urine), is the form which is normally analyzed. In suspected cases of congenital adrenal hyperplasia, the assay of an intermediate product, pregnanetriol, may be important in differentiating this disease from other adrenal cortical disorders.

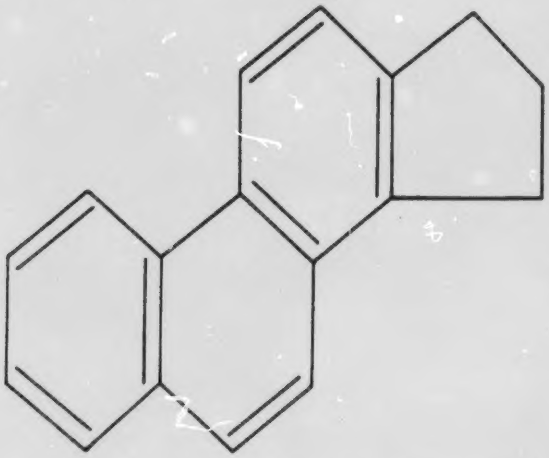


Figure 6. Structure of the cyclopentenophenanthrene ring.

6-5. *Androgens.* The testicular androgen, testosterone, is not normally excreted in the urine but is metabolized to androsterone, epiandrosterone, and 11-hydroxyandrosterone (the prefix *iso* is sometimes used instead of *epi*) which are normally found in the urine as sulfates and glucuronides. The remainder of the urinary androgens are androsterone and dehydroepiandrosterone of adrenal cortical origin. In males approximately one-third of the urinary androgens are formed in the testis and two-thirds are produced by the adrenal cortex. In females the androgens are formed solely in the adrenal cortex. Androgens are assayed clinically by the urinary 17-ketosteroid procedure. The method is not specific for androgens, however.

6-6. *Corticoids.* Referred to as hormones of the adrenal cortex proper, or corticosteroids, over 40 members of this group have been isolated, but only a few are biologically active. The corticoids help regulate protein, carbohydrate, and fat metabolism, and water and electrolyte balance. The above classes of hormones are formed from the same basic compounds (acetates) which form cholesterol. Cholesterol is thought to be converted to the adrenal cortical hormones by one of several complicated enzymatic pathways. These hormones are all basically similar and their separation sometimes depends upon lengthy extraction procedures with subsequent colorimetric analysis. Procedures for 17-ketosteroids and hydroxycorticosteroids are normally used to assay adrenal cortical hormones. Shown in table 5 is a list of steroids which are of particular interest in an evaluation of adrenal cortical function and which react in one of the common corticoid assays.

6-7. The procedure used for *17-ketosteroids* assays the androgens (androsterone, epiandrosterone, dehydroepiandrosterone) and the nonandrogen etiocholanolone by the Zimmerman reaction. In the Zimmerman reaction, 17-ketosteroids produce a red color in alkaline solution with m-dinitrobenzene. Urine sugar levels of more than 1+ will inhibit this reaction, causing low values. The 17-ketosteroids can be further divided into alpha and beta fractions, the beta-17-ketosteroids being precipitated by digitonin, whereas the

TABLE 5

STEROID HORMONES RELATED TO CORTICAL FUNCTION

Steroid	*Synonyms*		*Reaction*
Corticosterone		Compound B	
11-Dehydrocorticosterone		Compound A	
11-Dehydro-17-hydroxycorticosterone	Cortisone	Compound E	17-Hydroxy, 17-KG*
17-Hydroxycorticosterone	Hydrocortisone Cortisol	C-21 Compounds Compound F	17-Hydroxy, 17-KG
11-Deoxycorticosterone		Compound Q	
17-Dydroxy-11-Deoxycorticosterone		Compound S	17-Hydroxy, 17-KG
Cortol			17-KG
Cortolone			17-KG
Tetrahydrocortisone			17-Hydroxy, 17-KG
Tetrahydrohydrocortisone			17-Hydroxy, 17-KG
Pregnantriol			17-KG
Aldosterone			
17-Hydroxy Progesterone			17-KG
17-Hydroxy Pregnanolone			17-KG

*17-KG = 17-Ketogenic steroids analysis
17-Hydroxy = 17-Hydroxycorticosteroids

alpha are not. The alpha-17-ketosteroids normally predominate (85 to 95 percent), consisting of androsterone and etiocholanolone, while the beta fraction is present in only small amounts and consists of epiandrosterone and dehydroepiandrosterone. Normal urine values for neutral 17-ketosteroids are as follows. Children: daily output in the urine for children to 6 years of age is less than 1 mg. Values rise gradually until adult levels are reached at 12 to 18 years of age. Adult female: the usual range for the adult female is 5 to 15 mg./24 hours. Adult male: 8 to 25 mg./24 hours. Values for 17-ketosteroids are diminished in hypopituitarism, pituitary tumors, hypogonadism, Addison's disease, and certain other conditions. Values are elevated in cases of testicular tumors, adrenal hyperfunction, and following endocrine therapy. When you submit specimens for analysis, a 250 ml. aliquote of an entire 24-hour specimen is required. The specimen should be collected in a large container into which 10 ml. of concentrated hydrochloric acid have been placed before collection. This acidification during collection of the specimen is essential for the preservation of steroid constituents. As a precaution, tell the patient that the jar contains hydrochloric acid and stress the care necessary to avoid injury from the acid. The container must be conspicuously labeled with a danger sign stating the amount of hydrochloric acid it contains. If the container is obtained by ward personnel, they must also be given instructions as to the danger involved.

6-8. The Porter-Silber reaction for *17-Hydroxy-corticosteroids* is the most widely used. It is a reaction between steroids and a phenylhydrazine-sulfuric acid reagent to produce a yellow color. The steroids reacting account for about 30 percent of all the excreted C–21 steroid compounds. Either high protein or urine sugar levels greater than 1+ will interfere with the Porter-Silber reaction for 17-Hydroxy-corticosteroids. Hydrochloric acid is used as a preservative as was mentioned for ketosteroids in the previous paragraph.

6-9. **Pituitary Hormones.** The pituitary gland is a small (average 1.3 × 1.0 × 0.5 cm.), rounded grey body attached to the base of the brain. It is often referred to as the "master gland" because it regulates many other endocrine activities. The most important group of pituitary hormones in man are those which originate in the anterior lobe of the pituitary gland. Hormones of this group are known as the adenohypophyseal hormones. They include gonadotrophic hormone, thyrotophic hormone, adrenocorticotrophic hormone (ACTH), and somatotrophin. Only the first of these, gonadotrophic hormone, is commonly assayed directly in the clinical laboratory. The gonadotrophic hormone is responsible for activity of the ovaries in the female and spermatogenesis as well as androgen production in the male. Actually, the gonadotrophic hormone is a group of hormones. Included in the group are follicle-stimulating hormone (FSH), lutenizing hormone (LH), interstitial-cell-stimulating hormone (ICSH), and luteotrophic hormone (LTH). The assay of pituitary gonadotrophin is accomplished by using laboratory animals to show one or more of the following effects: (1) increase in weight of the uterus or ovaries, (2) production of corpora lutea, (3) increase in weight of seminal vesicles, and (4) effect upon estrus which is characterized by proliferation of the vaginal epithelium. The assay of chorionic gonadotrophin, which is secreted by the placenta, is discussed under the heading of pregnancy tests later in this course. Results of gonadotrophic studies are reported in mouse units. A *mouse unit* is the least amount of estrus-producing hormone which induces desquamation (sloughing) of the vaginal epithelium in a spayed mouse.

6-10. **Catecholamines and Vanyl-Mandelic Acid.** The beta catecholamines include two active components which have been isolated from the adrenal medulla. These two compounds are epinephrine (adrenalin) and norepinephrine (noradrenalin). Chemically the catecholamines have properties which are similar to alcohols, phenols, and amines. Norepinephrine stimulates the hypothalmus and the anterior pituitary. Epinephrine is similar in its pharmacologic and chemical properties to norepinephrine except that norepinephrine possesses a n-methyl group and does not produce the anxiety and apprehension caused by epinephrine. Epinephrine is used therapeutically as a vasoconstrictor, cardiac stimulant, to induce uterine contractions, and to relax bronchioles. The synthesis of epinephrine and norepinephrine in the body begins with two amino acids, phenylalanine and tyrosine. Epinephrine is produced by the methylation of norepinephrine. Catecholamines are metabolized principally via methylation to produce 3-methoxy-4 hydroxy derivatives. Upon oxidation, the methoxyamines produce corresponding acids. The major acid produced is 3-methoxy-4 hydroxy mandelic acid—also known as vanyl-mandelic acid (VMA).

6-11. Most laboratories prefer to measure VMA in the urine rather than the catecholamines because VMA is present in significantly greater amounts. There are two basic methods in general use for the measurement of VMA in urine. In the first method, an aliquot of the specimen is extracted with ethyl or amyl acetate. The extract is evaporated to dryness and the residue dissolved in water. This solution is made

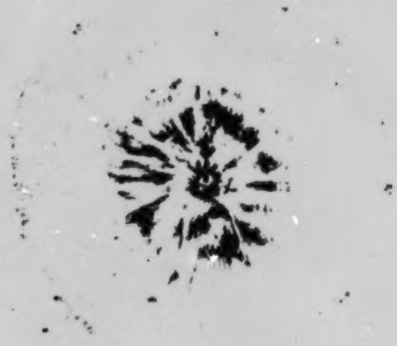


Figure 7. Section of urinary calculus showing concentric circles of salt deposition.

alkaline with K_2CO_3 and a color is developed with p-nitroaniline and ethanolamine in n-amyl alcohol. Normal values achieved by this method range from 1.8 to 10.8 mg. per 24 hours. In the second method, VMA is oxidized to vanillin which is then extracted and acidified. Vanillin is measured photometrically after color development with indol-phosphoric acid reagent. Normal values by the vanillin extraction procedure range from 0.7 to 6.8 mg. per 24 hours. Variations of these procedures are also used, at least one of which is available commercially in the form of a kit. Regardless of method, urine *must* be collected and maintained at a pH of less than 3.0 by the addition of acid to the collection container. Remember that appropriate warning must be given to the patient concerning concentrated acid. Certain medications, coffee, and some foods, particularly those which contain vanilla, are restricted for a period of 48 hours before collecting the specimen. The assay of VMA is performed to aid in the diagnosis of chromaffin cell tumors (so called because these cells stain readily with chromium salts), especially extra-medullary chromaffin tumors. Slight elevation of VMA may be observed in cases of malignant hypertension, but this is usually not to the extent observed with a tumorous condition.

7. Urinary Calculi

7-1. Analysis of calculi or "stones" from the urinary tract or gallbladder are usually performed in referral laboratories in the Air Force. The qualitative chemical tests are easy to perform, but require a number of reagents and manipulations. The frequency of calculus analysis is not sufficient to warrant providing the test in smaller medical units. However, technicians in referral laboratories should be aware of the general technique to better understand their responsibilities.

7-2. The incidence of urinary calculi in Americans is high in middle-aged and elderly individuals. Their occurrence in any group depends upon diet, immobilization (as in paralysis), a limited urine pH range, or lowered fluid intake and the presence of various salts which form the calculi. Calcium oxalate stones and mixed stones of calcium oxalate and the carbonate or phosphate salt are most commonly found. Next, magnesium ammonium phosphate stones are most frequent, while uric acid appears in about 5 percent of cases. Other compounds mentioned below are infrequently present. The nidus or initial substance is often organic in nature (epithelium,

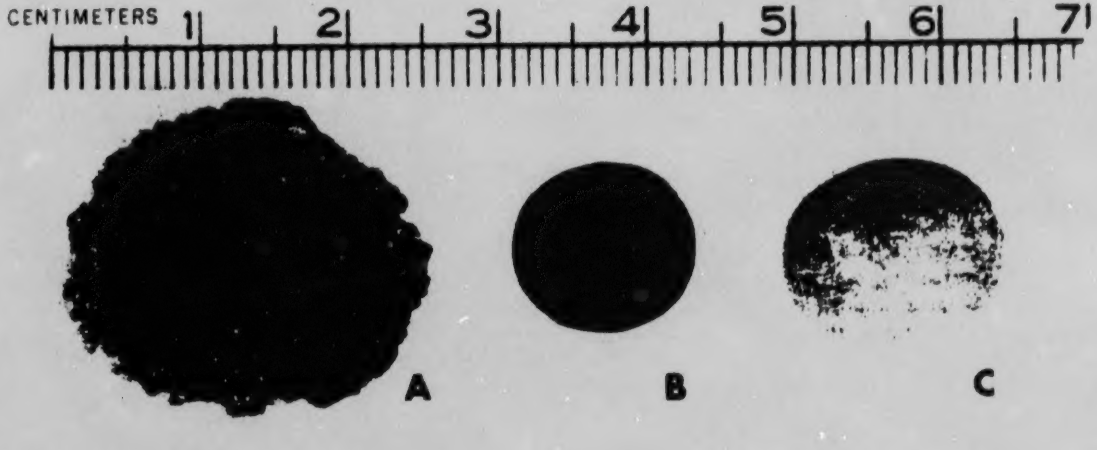


Figure 8. Relative size and physical characteristics of gall (a and b) and urinary calculus (c) stones.

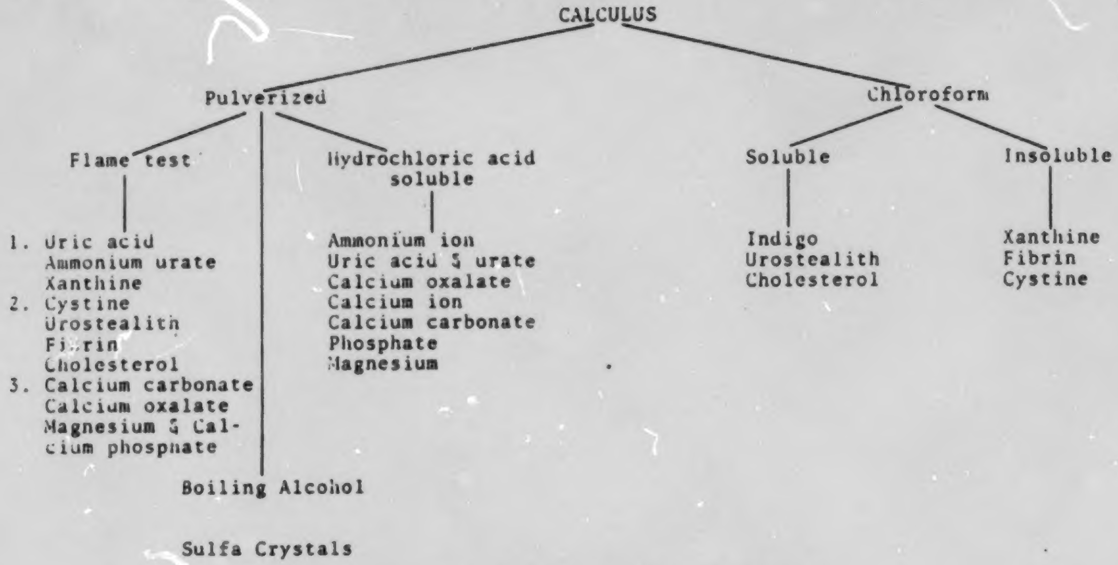

CALCULUS
Pulverized
Chloroform
Flame test
Hydrochloric acid soluble
Soluble
Insoluble
1. Uric acid
Ammonium urate
Xanthine
2. Cystine
Urostealith
Fibrin
Cholesterol
3. Calcium carbonate
Calcium oxalate
Magnesium & Calcium phosphate
Ammonium ion
Uric acid & urate
Calcium oxalate
Calcium ion
Calcium carbonate
Phosphate
Magnesium
Indigo
Urostealith
Cholesterol
Xanthine
Fibrin
Cystine
Boiling Alcohol
Sulfa Crystals

Figure 9. Outline for calculus analysis.

fibrin, bacteria). This nucleus can be seen in cross-sections of the calculus. Concentric circles of salt deposition may also be seen macroscopically. The nidus and concentric circles of deposition are pictured in figure 7. Under optimal conditions of salt concentration, pH, etc., successive deposits of salts are incorporated into a calculus around the nucleus material. Calculi vary in size from barely visible to as large as the calyx of the kidney. Smaller stones are passed in the urine but larger stones must be removed surgically. You can see the relative size and physical characteristics of two gallstones and one urinary calculus in figure 8.

7-3. Many schemata for chemical calculus analysis are available in the literature. An outline of a complete plan from AFM 160–49 is presented in figure 9. In this outline the stone is divided and one half is pulverized. Part of the pulverized material is subjected to a screening flame test which identifies the burning characteristics of certain groups of compounds. Then more specific qualitative tests, i.e., nesslerization for ammonium ion, phosphotungstic acid—uric acid, etc., identify definite chemical components. If sulfa drugs are present, they are dissolved with boiling alcohol, which is evaporated to dryness, and the crystals identified microscopically. Chloroform soluble portions of the remaining stone are tested for indigo, urostealith, and cholesterol; then any insoluble residue is examined for xanthine, fibrin, and cystine.

7-4. A chemical method is generally the best routine method for calculus analysis. Infrared spectroscopy and X-ray diffraction procedures have been used with limited success. X-ray diffraction is advantageous in the presence of uric acid and when the specimen is less than 5 mg. However, the cost of an X-ray diffraction instrument is prohibitive for most laboratories.

CHAPTER 4

Toxicology

POISONS HAVE ALWAYS been of wide interest, as reflected in many historical and literary accounts. During the time of the Athenian philosopher, Socrates (470–399 B.C.), juice of the hemlock shrub (*Conium maculatum*) was used as a poison to dispose of certain citizens. You may recall from history that Cleopatra, queen of Egypt until 30 B.C., supposedly died from the venom of an asp, and Napoleon is thought to have died of arsenic poisoning. Based on a literal (though perhaps incorrect) interpretation of Shakespeare, it is concluded that one of Shakespeare's characters was poisoned by the rather unlikely manner of having the poison poured into his ear while he slept. Recent studies of archeological material suggest that the decline and fall of the Roman Empire may have been caused by mass lead poisoning. The aristocracy used lead utensils for cooking and, as a result, may have suffered physical and mental deterioration.

2. In more modern times fact and fiction have been quite well separated, largely because of scientific laboratory analyses. It is now possible to detect poisons in the remains of persons who have been dead hundreds of years. The study of poisons and their effect on the human body is called toxicology. This is a sophisticated branch of clinical chemistry encompassing areas well beyond the scope of the average Air Force clinical laboratory. Isolation of poisons from biological material can be very difficult, particularly if they are present in low concentrations. There are so many different chemicals which could be considered that some systematic approach is necessary in the laboratory. Usually the average hospital laboratory tests for just a few specific substances ordered by a physician. If this is not the case, some useful method of laboratory classification is essential. One classification is the Gradwohl modification after Stas-Otto, in which poisons are categorized into six groups. Group I, volatile poisons, includes alcohols, cyanides, phenols, kerosene, etc. Group II, acid-ether extraction group, includes salicylates, barbiturates, etc. Group III, alkaline-ether extraction group, includes morphine, amphetamine, etc. Group IV, heavy metals, includes metals such as arsenic and mercury. Group V, gases, is comprised of compounds such as carbon monoxide. Group VI, miscellaneous, is made up of those which do not fit into any other group. Grouping provides a practical approach by which each of the various poisons may be isolated and separated. For example, the volatile group would be separated from other substances by steam distillation.

3. Only a few toxicology studies will be discussed in detail in this chapter, namely those having a very general application and usefulness. Familiarization with areas discussed here will prove of value at a time when implementation of seldom-used emergency procedures can prevent confusion and inaccuracy. However infrequently a procedure is performed, the technician should have a high degree of confidence and proficiency.

8. Routine Toxicology in Air Force Laboratories

8-1. Aspirin, alcohol, and carbon monoxide are so prevalent that every hospital laboratory should have chemistry procedures available to test for them. Toxicology tests provide the physician with information useful in one of three situations. First, it may be clinically desirable to establish therapeutic levels of certain drugs in the blood. In some instances the therapeutically effective level is close to the dangerous or toxic level. Hence, laboratory data must be accurate and timely. Second, diagnosis may depend upon ruling out the effect of drugs. For example, physiological or behavior changes due to a brain tumor, encephalitis, etc., can sometimes be confused with the effect of drugs unless the latter is ruled out or confirmed by the laboratory. Third, the possibility of accidental or intentional overdose of toxic substances may require treatment. Children frequently suffer accidental poisoning from drugs (or other chemicals) within their reach, especially aspirin. Also of particular interest to nearly all clinical laboratories is the blood alcohol which will be discussed at some length in this section.

8-2. **Medico-Legal Aspects of Blood Alcohol Tests.** One of the characteristically vague areas of the clinical laboratory is that which is concerned with the legal (forensic) aspects of laboratory procedures. Of the medico-legal problems which could involve the laboratory, proof of intoxication is one of the most likely. It is not uncommon for a commander to refer someone to the hospital for a *sobriety* evaluation. The term "sobriety" refers to a clinical opinion which only a physician should render. A blood alcohol test result is usually part of the contributing evidence in a sobriety evaluation; yet in reporting a blood alcohol test result, the technician is not stating whether the patient is or is not intoxicated. You should clearly understand the difference between a "sobriety" test and a "blood alcohol" test. The former implies clinical opinion which a laboratory technician is not professionally qualified to render. When you receive a request for a blood alcohol determination, you must be careful to observe precautions which are more or less unique to a legal situation. Sometimes, blood alcohol studies do not have a special legal meaning. For example, an unconscious patient may appear to be intoxicated when he is in reality suffering from injury or disease. In this case, the medical officer may request a blood alcohol test for diagnostic purposes. If possible, to resolve any question about the legal implications, the technician should communicate directly with the physician. If the case involves the rights of the patient as a citizen rather than purely diagnostic factors, certain considerations become significant. These considerations are discussed in the following paragraphs.

8-3. It may prove important that the request to perform the test is valid. Hospital policy makes it clear in most cases who may order laboratory procedures and how this is accomplished. Ordinarily a request does not have to be in writing. At the time a test is ordered, a medical officer will probably state whether or not the case has legal significance. If the results of the test are to be considered for admission as evidence in a court, it is vital to protect the rights of the individual. Rights include those guaranteed by the 5th Amendment of the Constitution and reiterated in Article 31 of the Uniform Code of Military Justice. These rights must not be disregarded. How much responsibility do you have in protecting the patient's rights? Should you draw the blood specimen and perform the test for which you have an order; or should you be concerned with legal responsibilities? Unfortunately, there is no simple answer to this question which can be set forth in a career development course. A technician is expected to follow all existing policies and directives as well as exercise good judgment commensurate with his training and experience. Law experts recognize that circumstances vary with each case, and this is, in fact, a reason for the time-consuming and expensive proceedings of military and civilian courts. The underlying reason, of course, is to protect both society and the rights of the individual. A technician who follows local policy and does not attempt decisions for which he has no authority is on safe ground. In some medical facilities it is policy for the person who draws the specimen to inform the patient of his rights under Article 31 of the UCMJ. Failure to do so would place laboratory evidence in a highly questionable light with regard to admissibility in court.

8-4. A laboratory employee should never draw blood against the patient's will unless he is specifically ordered to do so by competent authority. Then he should be aware of the patient's possible legal reaction to his act and be prepared to prove that drawing the specimen was legally acceptable and right. Insofar as possible, try to anticipate the various possibilities and find out in advance what is required. Those in a nonsupervisory capacity should be particularly careful to keep their supervisors informed of circumstances surrounding medical-legal situations. Supervisors will undoubtedly consult the Staff Judge Advocate in all cases of doubt.

8-5. When you draw a specimen, do not use alcohol as a skin cleansing agent (remember that all tinctures are alcoholic solutions by definition). If analysis is based on a reduction procedure, other reducing substances like acetone should not be used either. This is not so much a matter of probable contamination as one of technical doubt which may be raised in evaluating the results. You may use aqueous iodine or aqueous merthiolate before venipuncture.

8-6. Security is another consideration. The specimen must be labeled and secured so that it cannot be tampered with or confused with another specimen. A locked box which is kept in the refrigerator is useful for this purpose, provided access to it is controlled. It is usual procedure to maintain a *chain of custody receipt* which is a log showing how and by whom the specimen is handled from the time it is drawn until the test is completed. If the specimen is mailed, it should be sent by registered mail.

8-7. All readings and calculations must be checked and verified according to the existing policies of the laboratory concerned. Both a standard and at least one control must be run with a conscious effort to avoid cross-contamination. Records of the procedure should be kept to provide a concise and complete account of the analysis. You may need to refer to these records at a later date if you are called upon to testify in court.

8-8. Reports of a blood alcohol determination are to be kept within proper channels. Results are normally given only to a medical officer or to someone designated by the commander. Reports of a blood alcohol test are *never* released out of medical channels by laboratory personnel without specific authorization to do so. Careless handling of a report may result in considerable difficulty and compromise. Questions concerning disposition of reports are referred to the hospital registrar by the laboratory supervisor. The results should be clearly designated as mg./ml. or mg-%, properly dated, and signed. The time lapse between drawing a specimen and completing the test may in some instances affect legal acceptability of the results. We will discuss specimen stability later in this section. Keep in mind that any official report is a potential item of evidence in a court of law. Toxicology studies, and particularly blood alcohol tests, are the most likely items for legal consideration.

8-9. **Chemistry and Physiological Effects of Ethanol.** Ethyl alcohol, also known as grain alcohol or ethanol, has the relatively simple chemical formula C_2H_5OH. It has a toxic effect on various tissues of the body, especially the liver, if it is ingested in significant quantities over a prolonged period. Excessive intake in a short time may result in death. Ethanol is present in alcoholic beverages in concentrations varying from as little as 3 percent in some beers to approximately 50 percent in whiskies. A 50-percent solution of alcohol is termed 100 proof. As suggested in the chapter on gastric analysis, alcohol can be absorbed in the stomach through the gastric mucosa as well as in the small intestine. The blood level of alcohol rises more rapidly if the stomach is empty when the alcohol is consumed. Alcohol is distributed throughout the body and can be detected in tissue and body fluids, including spinal fluid. Less than 10 percent of the alcohol ingested appears in the urine, as it is steadily oxidized in liver and kidney tissue to carbon dioxide and water.

8-10. The metabolic process of breaking down alcohol is as follows. First, C_2H_5OH is transformed into acetaldehyde, CH_3CHO, which is then oxidized. Blocking the chemical oxidation of acetaldehyde at this point can be accomplished with drugs like disulfiram ("Antabuse"). The resultant accumulation of acetaldehyde causes extreme nausea and may be effective as a means of discouraging alcoholic intake. Acetaldehyde is normally converted to acetic acid, CH_3COOH, also known as ethanoic acid. Acetic acid then enters a metabolic cycle, resulting in final oxidation to carbon dioxide and water with the release of a significant number of calories. In the process of the breakdown of alcohol, other organic substances may be formed in small amounts which are destructive to kidney and liver tissue. Alcohol is excreted in the urine as noted above, but not to any great extent. Most of the alcohol in the blood is reabsorbed in the kidney tubules. Some is exhaled in vapor form from the lungs, a phenomenon which makes possible the so-called balloon test for analyzing the alcohol level of breath. The balloon test is rarely used any more as an index of intoxication, and it is not used at all in the Air Force.

8-11. It cannot be stated precisely at which level the person's physical or emotional condition changes. As a general rule, at a blood level of less than 50 mg-% (0.5 mg./ml.) a person is asymptomatic. Coordination, physical control, and emotional stability deteriorate as the blood level rises; at a blood concentration of 150 mg-% (1.5 mg./ml.) a person is considered "under the influence." Interpretation is, of course, a matter of clinical judgment as noted previously. Based on actual observations, approximately 50 percent of the population will show clearly observable signs of intoxication at a blood level of 150 mg-%. At least 80 percent of all individuals with a blood level of 200 mg-% appear to be intoxicated as the term is normally used. That is, their conduct and physical coordination are significantly affected. At blood levels of 300 mg. per 100 ml. of blood, few drinkers are able to control themselves. There is onset of coma at approximately 400 mg-%, from which some do not recover. Blood levels above 500 mg-% are nearly always fatal.

8-12. The effects of alcohol at levels less than those which are fatal are well known to most people. The overall effect is that of a depressant. Prolonged use of alcohol results in liver damage, commonly described as alcoholic cirrhosis. There are many associated symptoms of physical deterioration in chronic alcoholics, including vitamin deficiencies. Inability of the liver to excrete porphyrin in the bile may result in abnormal excretion of urinary porphyrins. Lipids in the urine (lipuria) may also result from alcoholism. In acute alcoholism, there is a moderate rise in the serum amylase level, as well as an abnormal glucose tolerance curve. A hypoxic condition known as histotoxic hypoxia may also result. This is characterized by lack of oxygen available to the tissues. Many symptoms of alcohol poisoning are similar to those resulting from other toxic substances such as lead, organic solvents, etc. Ethyl alcohol is least toxic of the alcohols.

8-13. Methyl alcohol (methanol, wood alcohol), CH_3OH, is far more toxic than ethanol. Blindness and death may result from any significant intake of methyl alcohol. There is very little physical difference between ethanol and methanol, which frequently results in the mistaken use of methanol. The two can be distinguished chemically, a problem which could be presented to a clinical laboratory if there is some question of whether a sample is methyl or ethyl alcohol.

8-14. **Laboratory Tests for Ethanol.** Alcohol is usually assayed in Air Force laboratories either by gas chromatography or by chemical tests, including those which use a diffusion principle and those which use an enzyme. Other means of analysis, including aeration and distillation, are rarely, if ever, applied. The Leifheit method, described in AFM 160–49, is based on the following principle: alcohol is diffused from the specimen into a solution of potassium dichromate in a diffusion chamber, shown in figure 10. The hexavalent chromium of dichromate is reduced by alcohol to trivalent chromium, resulting in a change of color from yellow to green. The intensity of the color developed in the acid dichromate is proportional to the concentration of alcohol. Reduction is illustrated as follows:

$$2K_2Cr_2O_7 + 8H_2SO_4 + 3C_2H_5OH \rightarrow 2K_2SO_4 + 3CH_3COOH + 11H_2O + 2Cr_2(SO_4)_3$$

Potassium dichromate-sulfuric acid solution is known as Ansties reagent. It should be stored in a brown, glass-stoppered bottle and handled with care. If a curve is used to calculate results, remember there will be some variation in O.D. readings each time Ansties reagent is prepared. For this reason it is preferred that a standard be run with each test. A standard is available through medical supply channels. A positive control may be prepared from dilutions of ethanol. A negative control is also run through the same procedure as the unknown by substituting distilled water for the blood specimen. Ansties reagent is used as a blank to obtain a 100-percent T reading with the spectrophotometer.

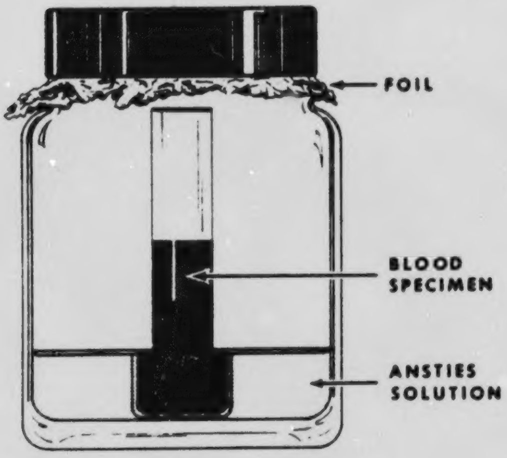


Figure 10. Diffusion chamber.

8-15. Alcohol is by no means the only substance which will reduce Ansties reagent. The presence of medications, ketone bodies, and lactic acid also reduces it. However, blood which reduces dichromate to the equivalent of 50 mg-% or more of ethanol almost certainly contains alcohol. The patient's plasma should be checked for acetone to rule out ketonemia before a specimen is reported as positive for alcohol. Blood does not normally contain alcohol and, therefore, readings equivalent to even 5 or 10 mg-% if reported would imply alcoholic intake. In practice it has been shown that values of this magnitude can be obtained by the dichromate method after the patient has eaten certain foods. By the same reasoning, it might be shown that values of 150 mg-% could actually be slightly less or slightly greater. To resolve this problem you would establish confidence limits using pooled serum containing alcohol. The criteria described in volume 1 for a quality control system apply very well in this instance. A competent laboratory technician would also recognize contamination as a source of error in this procedure, since many substances reduce dichromate. Contact between Ansties reagent and aluminum foil used to seal the chamber is a possible source of error. It is imperative that all glassware be chemically clean. Care is also required in handling the inner chambers (vials) which contain the blood. They must not be placed on a contaminated counter top and then immersed in the Ansties reagent. If there is any doubt about whether a particular substance will reduce dichromate, you can easily discover the answer by experimenting.

8-16. Whole blood is usually the specimen of choice, with lithium oxalate as the anticoagulant. If it is tightly stoppered, a blood specimen is known to be stable for at least 5 days under refrigeration. Deterioration of the blood may elevate the results because of the accumulation of reducing agents. Evaporation of alcohol will, of course, lower results. The diffusion chamber must be tightly covered to avoid loss of alcohol. (The inner vial is never covered.) An autoclave is usually used to facilitate the diffusion of the alcohol, but decompression must be slow enough to avoid boiling the Ansties reagent. The reaction mixture should then be allowed to cool before it is read in the spectrophotometer because optical properties of a solution vary with its temperature.

8-17. **Salicylates.** Aspirin, also known as acetyl salicylic acid, is the most frequently used of all medications. It is also the most common cause of poisoning in children between the ages of 1 and 4. Salicylic acid itself is too irritating to be used internally. Therefore, it is used therapeutically as derivatives of salicylic acid or organic acids. You can see that aspirin is an ester of acetic acid by examining figure 11. Compare the structure of salicylic acid with methyl salicylate (oil of winter green), well-known for its effects as a local irritant. Salicylates affect the central nervous system, specifically the hypothalamus, and by so doing control body temperature. The hypothalamus of the brain acts as a biological thermostat. Drugs, such as salicylates, which control this mechanism are called *antipyretics*. Salicylates also depress the central nervous system and thereby relieve pain. Hence, they are also termed *analgesics*. Salicylates have a significant stimulating effect on respiration, causing an increase in oxygen consumption and CO_2 production. Salicylates accomplish this by directly stimulating respiratory centers in the medulla.

8-18. If the intake of salicylates is excessive, the effects can be profound. Initially, there is respiratory alkalosis due to hyperventilation. This is characterized by an increase in the blood pH and a decrease in the pCO_2 of the plasma. Bases are then lost in the urine, causing the blood pH to return to normal. This is only a stage of the process and is referred to as "compensated respiratory alkalosis." Changes in acid-base balance described thus far are experienced even at therapeutic levels of salicylates. Persons with blood levels above 50 mg-% usually show toxic symptoms. Toxic doses cause increased pCO_2 of the plasma and increased loss of bicarbonates, thus shifting to a condition of metabolic

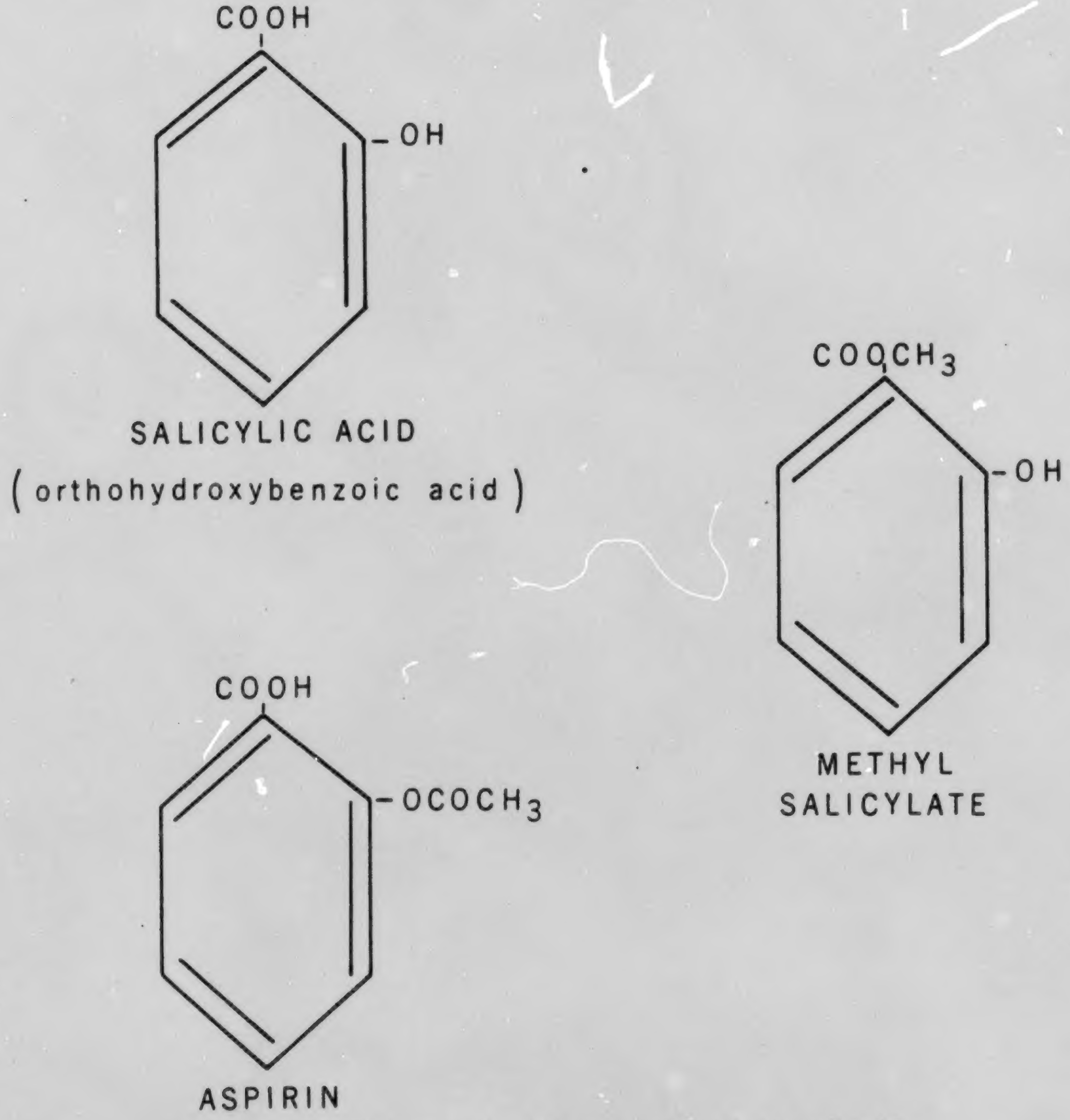


Figure 11. Structural formulas of salicylic acid, methyl salicylate, and aspirin.

acidosis. Impaired renal function caused by salicylates through vasomotor depression permits the accumulation of organic acids. Their effect on pH is in addition to the existing respiratory acidosis which results from CO_2 accumulation.

8-19. In summarizing the physiological response to salicylates, it can be said that there are two opposing effects. They are respiratory alkalosis from increased respiration and respiratory acidosis from increased CO_2 production. Metabolic acidosis from impaired renal function and loss of bicarbonate contribute to the problem of respiratory acidosis. Various metabolic effects of the salicylates are recognized with respect to the following metabolic activities:

a. Oxidative phosphorylation. Salicylates inhibit adenosine triphosphate (ATP) dependent reactions.

b. Carbohydrate metabolism. Many factors function here, in some cases lowering blood sugar levels, and in others causing hyperglycemia.

c. Nitrogen balance. There is a decreased synthesis and an increased breakdown of amino acids which result in a negative nitrogen balance.

d. Fat metabolism. Salicylates enhance the breakdown of tissue fatty acids and inhibit their synthesis.

e. Enzymes. Salicylates decrease the activity of a number of enzymes.

Besides the metabolic effects, salicylates affect endocrine functions, particularly the adrenal medulla, adrenal cortex, and the thyroid gland. Salicylates are usually used for analgesis or antipyresis, as previously suggested. Dangerously high blood levels lead to coma and death. Since the patient may appear only slightly affected during the first few hours following ingestion, laboratory studies may be important in diagnosis as well as in following the blood salicylate level.

8-20. **Laboratory Test for Salicylates.** In the most common laboratory procedure for salicylates, a red-purple iron complex is formed between salicylates and ferric nitrate in acid solution. The test is often performed upon an ethylene dichloride extract of serum, though either serum or urine may be used directly. A standard is prepared from salicylic acid. Acidified ferric nitrate produces a small amount of color with normal serum. The extent of this color may be determined with salicylate-free serum and subtracted from the O.D. reading of the unknown; or the standard may be prepared in salicylate-free serum. Normally, there are no salicylates in blood or urine. However, aspirin is so widely used it cannot be assumed that blood which may be available in the laboratory is free from salicylates. The specimen of choice is serum. Plasma may be used providing the concentration of sodium oxalate does not exceed 6 mg. per ml. The test described does not detect the acetylated form of salicylic acid. For this reason, aspirin cannot be used in preparing standards. The reaction measures free salicylic acid resulting from the body's conversion of acetyl-salicylic acid. The results are expressed as mg. sodium salicylate per 100 ml. of specimen because sodium salicylate is used as the standard. If it is necessary to report the result in terms of salicylic acid, the following conversions based on 160 and 138, the respective molecular weights of sodium salicylate and salicylic acid, can be applied:

$$\begin{array}{r}\text{mg. sodium} \\ \text{Salicylate/100 ml.}\end{array} \times \frac{138}{160} \text{ (or 0.86)}$$
$$= \text{mg. salicylic acid/100 ml.}$$
$$\begin{array}{r}\text{mg. salicylic} \\ \text{acid/100 ml.}\end{array} \times \frac{160}{138} \text{ (or 1.16)}$$
$$= \text{mg. sodium salicylate/100 ml.}$$

8-21. **Carbon Monoxide (CO).** More deaths are caused by carbon monoxide poisoning than by any other substance except alcohol. Pure carbon monoxide is flammable and colorless. It is also odorless in concentrations below 70 percent in the pure form. Faulty stoves and furnaces, and the internal combustion engine are the most common sources of accidental carbon monoxide poisoning. The possibility of carbon monoxide poisoning is always a factor of great importance in the investigation of aircraft accidents. It is inevitable that most Air Force medical laboratory technicians become involved in this problem at one time or another. Therefore, every technician must know at least the elementary aspects of carbon monoxide poisoning and how it is detected. When it is inhaled, carbon monoxide combines with hemoglobin to form carboxyhemoglobin. This imparts a bright, cherry-red color to the blood. Because hemoglobin has a much greater affinity for carbon monoxide than for oxygen, carbon monoxide replaces oxygen and thereby causes anoxia. It is possible to displace carbon monoxide which is attached to the hemoglobin molecule with oxygen; therefore, a person suffering from carbon monoxide poisoning may be revived if he is given oxygen before respiratory arrest and coma ensue.

8-22. Blood levels above 40 percent carbon monoxide are nearly always fatal. At blood levels less than 40 percent, symptoms may vary from a slight headache to vertigo, ringing in the ears, and severe head pain. There are usually no symptoms at blood levels less than 5 percent because of carbon monoxide in the atmosphere, 0 to 5 percent is considered normal. A heavy smoker's blood normally contains about 8 percent carbon monoxide. For this reason his blood provides an ideal high normal control on CO analyses. Children and individuals with a low hemoglobin are more susceptible to carbon monoxide poisoning than are healthy adults. Both the concentration of the gas in the atmosphere and the time of exposure are also factors to be considered in predicting the physiological response.

8-23. **Determination of Carbon Monoxide in Blood.** As stated in the preceding paragraph, the cherry-red color of blood suggests carbon monoxide poisoning. The actual blood level may be determined by any one of several methods, the most reliable being gas chromatography. There are also various screening tests in use, the simplest being a dilution test for the detection of carboxyhemoglobin. In this procedure, the blood is diluted with water so the solution appears a faint pink. A solution of 20 percent NaOH is then added and quickly mixed. If the blood contains less than 20 percent carboxyhemoglobin, the pink color rapidly disappears and the solution becomes yellow. Persistence of the pink color for more than a few seconds indicates carbon monoxide levels above 20 percent. This test is simple and specific for carbon monoxide. However, the time element is critical. A delay in color change from pink to yellow *after more than a few seconds* following addition of the NaOH is considered a *negative* test. A negative control should be run for contrast.

8-24. Another common and somewhat more difficult screening procedure is the palladium method. Carbon monoxide, acid-liberated from a blood sample, reacts with palladium chloride in a microdiffusion cell, causing the release of metallic palladium which forms a "mirror" on the surface of the reagent. The palladium procedure is outlined in detail in AFM 160–49. Methods using the spectrophotometer have also been devised.

8-25. A spectrophotometric method may be used for determining carbon monoxide which involves sodium hydrosulfite reduction of a blood sample which has been diluted with 0.4 percent ammonia. Oxyhemoglobin is completely reduced in the presence of sodium hydrosulfite, whereas carboxyhemoglobin is not reduced. The optical density quotient is determined from readings at two different wavelengths and the percentage of carboxyhemoglobin is then read from a prepared curve. Whenever it is necessary to prepare a standard curve, carbon monoxide may be generated and used to saturate a blood sample from which dilutions can be prepared. Generation must be carried out in a hood, using all necessary precautions. Keep in mind that *carbon monoxide is dangerous.* A gas generator is set up by mounting a separatory funnel containing formic acid (85 to 90 percent) over a vented Erlenmeyer flask containing concentrated sulfuric acid maintained at 60° to 70°C. The formic acid is allowed to drop into the sulfuric acid slowly, and the resultant gas is passed first through distilled water to remove impurities, then through anhydrous calcium chloride to dry, and finally, into the flask containing the blood to be saturated.

8-26. In addition to the methods described, both the Van Slyke apparatus and the Natelson microgasometer may be used for carbon monoxide studies. Regardless of method, the specimen to be used is oxalated blood. It is usually stable until grossly hemolyzed or deteriorated. A few days' delay, as may be encountered in mailing the specimen, should not affect the results significantly. Confirmation of results by a reference laboratory may be desirable, especially after aircraft accidents and in medico-legal cases.

9. Special Toxicology Studies

9-1. The classification presented in this section is a very brief synopsis of the Gradwohl modification and by no means adequately covers the complex science of toxiocology. Students who desire more detailed information may refer to a textbook of toxicology. Qualitative differentiation of toxic substances must not be oversimplified, especially if the analysis is performed on body tissue instead of on an isolated chemical. Interferences and lack of specificity can easily lead to erroneous conclusions when toxicology tests are performed by inexperienced personnel.

9-2. **Volatile Poisons.** On occasion, even the small Air Force laboratory may be called upon to perform toxicology tests for volatile substances other than alcohol or refer them to a consultant laboratory. It is sometimes necessary to prepare an acid distillate such as is needed for rapid spot tests. A portion of tissue (e.g., 100 g. of brain) is homogenized in a cold Waring blender. The homogenate is then transferred to a Florence flask together with a rinse of approximately 100 ml. of distilled water. To this mixture 225 g. of ammonium sulfate and 5 ml. concentrated sulfuric acid is added. The flask is connected to the distillation apparatus illustrated in figure 12.

a. Volatile reducing substances. The purpose here is usually to rule out the presence of substances which might give a false-positive test for ethanol. Anstie's reagent will react with ethanol, methanol, acetaldehyde, formaldehyde, and acetone. Procedures for differentiation can be found in a textbook of toxicology.

b. Cyanides and sulfides. Cyanides may be absorbed in alkali and converted to cyanogen chloride. The cyanogen chloride is reacted with methyl-phenyl pyrazalone to form a blue color.

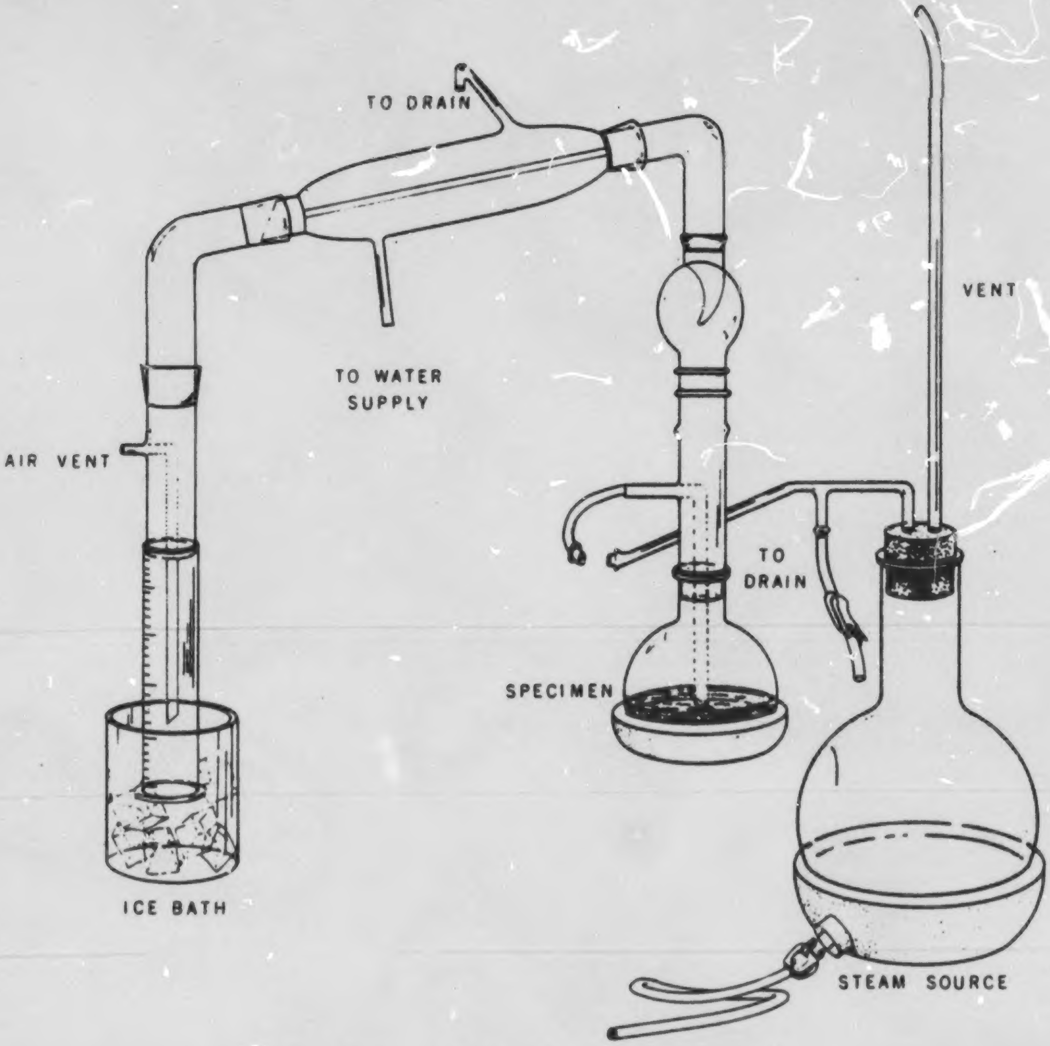


Figure 12. Distillation apparatus.

Sulfides may also be detected by conversion to bismuth sulfide or by the formation of a color complex with picric acid. If 5 ml. of acid distillate are made alkaline with sodium hydroxide, the presence of cyanide can be detected with a few drops of saturated picric acid. The appearance of a red color when the mixture is heated gently for 10 minutes is considered a positive test.

c. Halogenated hydrocarbons. This group includes carbon tetrachloride, trichloroethylene, and chloroform. They may be separated by a microdiffusion principle. A popular means of detecting halogenated hydrocarbons involves the Fujiwara reaction. In this reaction 5 ml. of acid distillate are added to 1 ml. of redistilled pyridine. After the addition of 1 ml. of 10 percent NaOH, the mixture is boiled for a few minutes. If chlorinated hydrocarbons are present which have more than one chlorine atom, a pink color develops within 1 to 3 minutes. The sensitivity of this procedure in detecting quantities in the distillate is as follows:

Carbon tetrachloride	0.10%
Trichloroacetic acid	0.05%
Trichloroethylene	0.01%
Chloral hydrate	0.005%
Chloroform	0.04%

Both positive and negative controls should be run.

d. Phenols. Chemicals with the characteristic phenol structure may be detected by the formation of insoluble tribromphenol. In one test, sufficient saturated bromine water is added to 5 ml. of acid distillate. In the presence of phenols, a rather heavy yellow to white precipitate forms.

The test detects as little as 0.002 percent phenol and 0.02 percent naphthol in the distillate. Other phenols are also detected, including cresol.

9-3. **Barbiturates, Acetanilid, and Phenacetin.** Barbiturates are widely used as prescription drugs to depress the central nervous system. After acid-ether extraction, barbiturate derivatives may be identified based on their reaction with cobalt acetate and isopropyl amine. Phenacetin (acetophenetidin) and acetanilid have analgesic and antipyretic activity. Phenacetin is an ingredient in APC compound (which also contains acetylsalicylic acid and caffeine). Both give a positive indophenol test and can be distinguished by means of the isonitrile test, which is positive for acetanilid but not for phenacetin.

9-4. **Alkaloids.** This group can be extracted with alkaline-ether and includes strychnine, amphetamines, morphine, and many others. Differentiation is quite involved and difficult. However, there is a rapid test which may be performed on urine to detect large doses of alkaloids in general. A reagent for this purpose is prepared as follows.

Mercuric iodide (red)	3 g.
Potassium iodide	2 g.
Glacial acetic acid	20 ml.

q.s. to 60 ml. with distilled H_2O

Five to 10 drops of the mercuric iodide reagent are added to a few milliliters of urine in a test tube. The immediate appearance of a slight turbidity means there are either albumin or alkaloids present. The tube is heated gently for differentiation. If turbidity is due to alkaloids, the precipitate completely dissolves upon heating.

9-5. **Heavy Metals.** Each of the heavy metals is tested for separately. Most significant are arsenic, mercury, and lead. Of these, lead may have special significance to environmental health laboratories. Lead is a very common metal used in petroleum additives, storage batteries, ammunitions, etc. You are encouraged to investigate procedures for the determination of heavy metals and also the special instructions issued by reference laboratories regarding referral of specimens for heavy metal studies. Special attention is necessary to avoid contamination in the case of urinary lead studies.

9-6. **Tissue.** Tissue specimens should be frozen solid in dry ice. Great care must be taken to avoid contaminating tissue specimens with formaldehyde, as it interferes with various toxicological studies. Specimens which may be submitted for study include bone marrow, hair, brain, muscle, and, in fact, practically any body tissue or body fluid. If fluid is aspirated into a container at autopsy, the container and apparatus must be free from interfering contaminants. Specimens taken should be adequate. Remember, it is always better to submit too much specimen to a consultant laboratory than too little. A list of suggested specimens and the amount to submit for analysis is presented in table 6. These and other toxicological specimens may be forwarded to the United States Air Force Epidemiological Laboratory, Air Force Systems Command, Lackland Air Force Base, Texas. If more practicable, specimens may also be sent to the appropriate regional laboratory having the capability to perform toxicological studies. In addition to the specimen, a pertinent history is required. This could save many days of unnecessary testing. Tissue specimens are best preserved, with few exceptions, with dry ice. If an autopsy is performed, one-half brain specimen, 500 g. liver, one whole kidney, and all available blood and urine must always be forwarded. For additional instructions consult AFR 161–17, *Environmental Health, Forensic Toxicology, and Radiological Health Professional Support Functions.* In cases of aircraft accident fatalities, all facilities must send fixed and frozen specimens for toxicological examination directly to the Armed Forces Institute of Pathology, Washington, D.C. in accordance with AFR 160–55, *The Armed Forces Institute of Pathology and Armed Forces Histopathology Centers.*

10. A Review of the Actions of Poisons

10-1. As you know from the previous discussion and from your own experience, a poison is a substance which may produce death, serious illness, or harmful effects when it is introduced into the body in a relatively small quantity. In concluding this chapter, let us briefly consider some basic concepts in toxicology, particularly with reference to the general effects of poisons on the body. We have already considered in some detail the specific effects of such poisons as alcohol, carbon monoxide, and salicylates.

10-2. The effects of poisons may be local or remote and some poisons have both a local and remote effect. *Local effect* means direct action on the part to which the poison is applied, such as corrosion and irritation; *remote effect* means the action of the poison on some organ remote from the seat of application or point of introduction. Sometimes a poison shows no effect or only a slight one, until several doses have been taken. Then an effect is produced which nearly equals that produced by taking the whole amount at one time. This is known as *cumulative effect.*

10-3. The effect of a poison depends not solely on its concentration, but its solubility, the method of its introduction into the body, and

TABLE 6

COLLECTION AND SUBMISSION OF TOXICOLOGICAL SPECIMENS

Poison Suspected	*Specimen*	*Minimum Quantity*
Alcohol	Blood; brain	10 ml; 500 grams
Alkaloids	Urine	250 ml
Antihistamines	Urine	250 ml
Barbiturates	Urine; serum	250 ml; 10 ml
Lead	Urine; kidney	250 ml; 500 gm
Mercury	Kidney	1 kidney
Arsenic (chronic)	Hair	5 gm
Arsenic (acute)	Stomach contents; vomitus	All available
Other metals	Stomach contents; vomitus	All available
Volatile poisons	Brain	500 gm
Inhaled poisons	Lung	1 lung
Unknown	Vomitus	All available
	G.I. Contents	Stomach, small intestine
	Liver	500 gm
	Kidney	1 kidney
	Blood	50 ml
	Urihe	All available
	Brain	1 lobe
Bromides	Serum	5 ml
Salicylates	Serum	5 ml
Cyanides	Stomach contents	All available
Fluorides	Stomach contents; urine; vomitus	All available
Carbon Monoxide	Heparinized blood	10 ml
Meprobamates	Urine	100 ml
Drowning	Right atrial blood	10 ml
	Left atrial blood	10 ml
	The drowning water	20 ml

the rapidity of its absorption into the system. The method of introduction may determine its toxicity. For example, snake venom taken into the mouth and perhaps even into the stomach during first-aid treatment of snakebite is not ordinarily harmful, but snake venom injected hypodermically is extremely poisonous.

10-4. There are various ways in which poisons may be introduced into the body, the most common being by mouth, inhalation, and injection. Poisons taken by mouth enter the circulation through absorption from the stomach and intestine and those inhaled enter the circulation through the air passages and lungs. When they are introduced by hypodermic injection, poisons enter the circulation through absorption from the body tissues. If the injection is intravenous, the poisons are introduced directly into the bloodstream. Poisons may also be introduced by application to open wounds and to the unbroken skin. After entering the circulation, a poison is carried by the blood to the tissues and organs susceptible to its action.

10-5. Most of the elimination of poisons from the body takes place in the kidneys, lungs, liver, gastrointestinal tract, skin, and salivary glands. Poisons may be excreted from the system unchanged or in the form of other compounds into which they have been transformed by the action of the various body organs and tissues. The most damaging effects of some poisons are found at the points of excretion, as in the kidneys and colon in poisoning by mercuric chloride (bichloride of mercury).

10-6. Various conditions of the individual may modify the actions and effects of poisons on the body. The age of the person makes a great deal of difference, young children being far more susceptible to poisons than adults. Conditions caused by poisons vary because of personal idiosyncrasy; that is to say, some persons by nature are unusually sensitive to certain poisons, while others possess a natural tolerance for certain poisons that is not the result of habitual use. Through habitual use of certain poisons, especially the narcotics, most persons may become so accustomed to their effects that they are not poisoned when taking doses that would ordinarily prove lethal in the unaddicted. It occasionally happens, however, that continual external use of chemical substances results in hypersensitivity.

10-7. The actions of poisons may be considerably modified by disease, some diseases increasing and others lessening the action of poisons. In the latter case, large doses are usually required to produce the desired effect.

10-8. Poisoning may be either acute or chronic. *Acute poisoning* is the condition brought on by taking one overdose of a poison. *Chronic poisoning* is the condition brought on by taking repeated doses of a poison or as the result of the absorption of the poison over a long period of time. Many industrial workers, instrument assembly technicians, fuel handlers, and painters are some of the occupational groups subject to chronic poisoning. You, as a laboratory technician, are also exposed to a variety of poisonous chemicals.

10-9. Poisons may be classified according to their mode of action in a manner similar to the presentation in Section 9 of this chapter. Although of limited use in the clinical laboratory, a clinical classification is helpful because it describes how poisons act. For example, poisons may be described under the headings of corrosives, irritants, neurotics, and gases.

10-10. **Corrosives.** Corrosives are substances which rapidly destroy or decompose the body tissues at point of contact. When they are ingested, there is immediate burning pain in the mouth with severe burning pain in the esophagus and stomach. Swallowing is very difficult, respiration is impeded, and vomiting is inevitable. Examples are strong acids and alkalis.

10-11. **Irritants.** Irritant poisons are those agents which do not directly destroy the body tissues but set up an inflammatory process at the site of application or contact. Some examples are potassium nitrate, zinc chloride, zinc sulfate, ferrous sulfate, silver nitrate, arsenic, iodine, and phosphorus. General symptoms include nausea, vomiting, and purging (frequently the vomited matter and stools contain blood), and pain and cramps in the abdomen. In some cases, there is inflammation of the urinary tract.

10-12. **Neurotics.** Neurotics are poisons which act on the brain, spinal cord, and the central nervous system. Some examples are opium, hydrocyanic acid (prussic acid), ether, chloroform, aconite, strychnine, belladonna, ethyl and methyl alcohol, LSD, and the barbiturates. Symptoms may be divided into two subclasses:

a. Depressants, which produce symptoms characterized by a period of exhilaration followed by drowsiness and stupor, slow and stertorous breathing, cold, clammy skin, cyanosis, slow pulse, muscular relaxation, dilated or contracted pupils, and insensibility to external impressions.

b. Excitants, which produce symptoms characterized by rapid and feeble pulse, delirium, hot and dry skin, a sense of suffocation and the inability to breathe, shuddering and jerking of muscles, dilated or contracted pupils, disordered vision, and sometimes convulsions and tetany (as in the case of strychnine poisoning).

10-13. **Gaseous Poisons.** These are poisons present in the gaseous state which, if inhaled, destroy the oxygen-carrying property of the blood. They irritate the tissues of lungs and air passages, and, if in contact with the skin or mucous membranes, are highly irritating. Symptoms include irritation and corrosion of the respiratory tract, with resultant bronchitis, and irritation of the eyes, mouth, stomach and kidneys.

10-14. **Food Poisoning.** This chapter would be incomplete without at least mentioning food poisoning. You will, of course, study this topic in greater detail in a subsequent volume relating to microbiology. Food poisoning can cause acute attacks of illness in more men in a short time than any other agent. The term "food poisoning" is conventionally divided into two types, food intoxication and food infection.

a. Food intoxication is due to a specific toxin produced outside the body, for example, the toxin of botulism. Other organisms also cause food intoxication by producing toxins, the exact nature of which is imperfectly understood. These toxins are formed under suitable conditions usually by Staphylococcus, occasionally by Streptococcus, and rarely by coliform and Proteus species.

b. Food infection usually is caused by a specific group of organisms, namely the Salmonella group, and occasionally by the dysentery group. Gastrointestinal distress, nausea, vomiting, diarrhea, urticaria, and circulatory and nervous disturbances are the general symptoms of food poisoning and they may vary from mild discomfort to violent disturbances of normal functions of the body. In more severe forms the neurologic symptoms may overshadow the gastrointestinal symptoms, followed by relapse. Death is usually due to respiratory paralysis, cardiac failure, or secondary pneumonia.

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Study Reference Guide

Chapter Review Exercises

Answers for Chapter Review Exercises

Volume Review Exercise

ECI Form No. 17

STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 300 through 311

Guide Number	
300	Introduction to Kidney Function Tests; Retention and Excretion Tests; pages 1-4
301	Clearance and Concentration-Dilution Tests; pages 4-9
302	Introduction to Gastric Analysis; Basic Anatomy and Physiology of the Stomach; pages 10-13
303	Laboratory Procedures in Gastric Analysis; pages 13-14
304	Introduction to Special Chemistry Tests; Referral Chemistries: General; Electrophoresis; pages 15-19
305	Referral Chemistries: Chromatography; Serum Iron and Iron-Binding Capacity; Protein-Bound Iodine; pages 19-22
306	Hormones; pages 22-26
307	Urinary Calculi; pages 26-27
308	Introduction to Toxicology: Routine Toxicology in Air Force Laboratories: General; Medico-Legal Aspects of Blood Alcohol Tests; Laboratory Tests for Ethanol; pages 28-31
309	Routine Toxicology in Air Force Laboratories: Salicylates: Chemistry and Physiological Effects of Ethanol; Laboratory Test for Salicylates; Carbon Monoxide (CO); Determination of Carbon Monoxide in Blood; pages 31-34
310	Special Toxicology Studies; pages 34-36
311	A Review of the Actions of Poisons; pages 36-38

CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objective: To demonstrate an understanding of the purpose, principle, procedure, and calculation for each of the common kidney function tests.

1. List four major functions of the kidney. (Intro.-1)

2. In which part of the kidney does most of the selective reabsorption occur? (Intro-1)

3. List three major physiological factors which play a role in normal kidney activity. (Intro.-2)

4. List four major categories of kidney function tests. (Intro.-3)

5. Describe the action of urease on urea. (1-2)

6. What is the purpose of SPS in the BUN method of Gentzkow and Masen? (1-4)

7. *a.* In the BUN procedure using Nessler's reagent, what chemical constitutent reacts with mercuric iodide to produce a color?
   b. What is the most probable cause of turbidity in the reaction mixture?
   (1-5)

8. Why is a Somogyi filtrate suggested as preferable to a TCA filtrate in the BUN procedure of Gentzkow and Masen? (1-5)

9. *a.* Where does uric acid come from in the body?
   b. What does an elevated serum uric acid level indicate?
   (1-7)

10. *a.* What is the principle of the phosphotungstic acid procedure for uric acid by the method of Henry, et al. as described in this text and in AFM 160-49?
    b. What is the normal serum uric acid level?
    c. Of what clinical significance is a decreased serum uric acid?
    (1-7, 8)

11. What is the general chemical nature of creatinine?(1-9)

12. *a.* Distinguish between creatinine and creatine.
    b. How is creatine assayed?
    (1-11)

13. Why is it unlikely that a patient would receive an overdose of PSP dye?(1-13)

14. What is the purpose of clearance tests?(2-1)

15. *a.* Calculate percent (average normal) urea clearance from the following data. Urine nitrogen level = 50 mg-% BUN level = 0.1 mg./ml.
    b. Why was the urine volume not specified in this problem?
    (2-3)

16. Calculate maximum urea clearance, C_m, from the following data.
    Urine urea nitrogen level = 90 mg./ml.
    Blood urea nitrogen level = 10 mg./ml.
    Urine volume = 100 ml. in 30 min.
    Patient is a child, 3′9″ in height.
    Is the result you calculate normal or abnormal?(2-8)

17. Calculate the maximum urea clearance (C_m) from the following data.
    Urine volume = 75 ml. in 30 min.
    BUN level = 0.08 mg./ml.
    Urine urea nitrogen level = 2.5 mg./ml.
    Patient's height = 5′9″.
    Is this normal?(2-8)

18. Calculate the standard urea clearance (C_s) from the following data.
    Urine urea nitrogen level = 1.6 mg./ml.
    Volume of urine = 50 ml. in 30 min.
    BUN level = 40 mg-%.
    Patient is 6′2″ in height.
    Is your answer normal? (2-8)

19. *a.* Calculate minimal urea clearance from the following data.
    Urine volume = 10 ml. in 30 min.
    Urine urea nitrogen level = 6.0 mg./ml.
    BUN level = 0.20 mg./ml.
    b. Is this normal?
    c. Do you consider this result highly significant? Why or why not?
    (2-8)

20. *a.* How would you describe a normal Mosenthal concentration test?
    b. How would you describe a normal dilution test following an intake of 1500 ml. of water?
    (2-17, 19)

CHAPTER 2

Objective: To identify terms relating to the basic anatomy and physiology of the stomach; to outline the basic steps of a gastric analysis; and to demonstrate a knowledge of tubeless gastric studies.

1. Define each of the following.
    a. Peristalsis
    b. Gastric pits
    c. Fundus
    d. Lamina propria
    e. Pyloric sphincter
    f. Parietal cells
    g. Columnar epithelium
    h. Autolysis
    i. Chief cells
    j. Rugae
    (3-2–4)

2. Explain how HCl is formed in the stomach. (3-6)

3. What is the average pH of gastric juice? (3-6)

4. Define each of the following.
   - *a.* Vagus
   - *b.* Lipase
   - *c.* Hypoacidity
   - *d.* Gastrectomy
   - *e.* Carbonic acid
   - *f.* Rennin
   - *g.* Hyperacidity
   - *h.* Achlorhydria
   - *i.* Pepsin

   (3-5–9)

5. *a.* How would you define *a degree* with reference to gastric acidity?
   b. How does this value relate mathematically to mEq/L?
   c. What is the normal total acidity in a fasting gastric specimen?
   (3-8, 9)

6. Sketch three approximation curves showing the relative responses to gastric stimulation in hyperchlorhydria, hypochlorhydria, and achlorhydria. Plot degrees of acidity versus time. (3-10–13)

7. What is the normal color of gastric contents? (4-2a)

8. How would accumulated blood from an ulcer be recognized? (4-2f)

9. Explain the titration procedure and calculation for combined acidity. (4-3)

10. If 2.5 ml. of gastric specimen titrates to the salmon-pink endpoint of Topfer's reagent with 0.5 ml. of 0.1*N* NaOH, how many degrees of free HCl are present? (4-3)

11. Name the indicator used to detect free HCl. (4-3)

12. How would you define combined gastric acidity? (4-3)

13. *a.* How would you describe the color of the endpoint in titrating for free acidity?
    b. For combined acidity?
    (4-3)

14. *a.* How is a tubeless gastric analysis accomplished?
    b. Of what value is this procedure?
    (4-4)

CHAPTER 3

Objective: To demonstrate an adequate familiarity with special tests and special techniques not normally carried out in small medical facilities.

1. What is electrophoresis? (5-3)

2. What happens to a protein fraction in an electrophoretic system at its isoelectric point? (5-4)

3. What two factors determine the rate and direction of migration of a partical in electrophoresis? (5-4, 5)

4. Name the serum protein fraction in order of fractionation from the positive to negative electrodes on an electrophoretic strip. (5-6)

5. Name three conditions in which electrophoresis is clinically useful. (5-7)

6. What is ferrohemoglobin solubility used for? (5-8)

7. What are the advantages of cellulose acetate as a medium in electrophoresis. (5-10)

8. Define chromatography. (5-12, 13)

9. What is serum (plasma) iron? (5-15)

10. What is the advantage in using hot trichloroacetic acid in the procedure for serum iron? (5-16)

11. What aspects of quantitation do all methods for PBI have in common? (5-18)

12. What is the purpose of treating serum for PBI with ion exchange resins? (5-19)

13. State the reaction involved in quantitating iodine by the ceric-arsenious acid reaction. (5-22)

14. Name at least five sources of contamination in the PBI analysis. (5-23, 24)

15. What is a hormone? (6-1)

16. How are estrogens usually assayed? (6-3)

17. Where is progesterone produced in the body? (6-4)

18. What are hormones of the adrenal cortex properly called? (6-6)

19. What is your responsibility as a medical technician in the collection of a 24-hour urine specimen for steroid analysis? (6-7)

20. Name at least three tissues which secrete hormones. (6-1–5, 9)

21. What is a *mouse unit* in the bioassay of gonadotrophin? (6-9)

22. How are steroids and VMA preserved in a 24-hour urine collection?(6-7, 8, 11)

23. Why are calculi usually analyzed at reference laboratories?(7-1)

CHAPTER 4

Objective: To demonstrate a knowledge of the properties of, and tests for, toxic substances commonly encountered in a hospital situation, and some of the medico-legal problems associated with handling toxicology specimens.

1. *a.* Explain the difference between a sobriety determination and a blood alcohol test. (8-2)
   b. If you are asked to inform an individual of his rights to avoid self-incrimination before you draw a blood alcohol specimen, where would you find a concise statement of these rights?(8-3)

2. May you use tincture of merthiolate to prepare the site of venipuncture when you draw a blood alcohol specimen? Why or why not?(8-5)

3. What is a chain of custody receipt? Why is it important?(8-6)

4. Describe the metabolism of ethanol. (8-10)

5. What are some of the physiological effects of alcoholism?(8-12)

6. *a.* How would you describe the basic chemical and physiological differences between ethanol and methanol?
   b. What is the percentage concentration of alcohol in 50 proof whiskey?
   (8-9,13)

7. *a.* What is the principle for the Leifheit (autoclave) method for the determination of blood alcohol?
   b. Referring to the equation for dichromate reduction, which reaction product is responsible for the color?
   (8-14)

8. *a.* What is the chemical nature of Anstie's reagent?
   b. What is it used for?
   (8-14)

9. Name two substances found in blood besides drugs which could interfere with the reduction of Anstie's reagent. (8-15)

10. Why should Anstie's reagent cool to room temperature before the cuvette is placed in the spectrophotometer? (8-16)

11. How long and under what circumstances can you estimate the stability of a blood sample for blood alcohol determination? (8-16)

12. *a.* Aspirin is described as an ***antipyretic*** and as an ***analgesic.*** What do these terms mean?
    b. How are salicylates able to control body temperature?
    (8-17)

13. *a.* Describe the chemical nature of aspirin.
    b. How can salicylates be tested for in the laboratory?
    (8-17, 20)

14. *a.* Describe the effect of salicylates on blood pH.
    b. List some metabolic activities of the body which are affected by salicylates.
    (8-18–20)

15. Explain why aspirin cannot be used as a standard in the ferric nitrate method for salicylates. (8-20)

16. Convert a salicylate level of 40.0 mg-% to its equivalent in terms of salicylic acid. (8-20)

17. *a.* What are the physical properties of carbon monoxide?
    b. Explain why carbon monoxide is harmful if inhaled.
    (8-21)

18. *a.* List three factors which determine how harmful exposure to carbon monoxide may be.
    b. What is the normal CO level of blood?
    (8-22)

19. *a.* Describe the principle of the palladium procedure for CO determination.
    b. What effect does sodium hydrosulfite have on oxyhemoglobin?
    c. Name two chemicals which may be reacted to produce carbon monoxide in the laboratory.
    (8-24, 25)

20. A physician requests that a blood specimen be mailed for a carbon monoxide test (your laboratory does not have a carbon monoxide procedure). How reliable would you consider the result after the specimen remains at room temperature in the postal system for 3 days? (8-26)

21. Describe a method for preparing an acid distillate such as used for spot tests. (9-2)

22. *a.* In the rapid test described for detecting urinary alkaloids, what other abnormal constituent besides alkaloids gives a positive test?
    b. How may these two substances be distinguished?
    (9-4)

23. To which of the chemical groups of toxic compounds discussed do each of the following belong?
    a. Strychnine
    b. Amphetamine
    c. Chloroform
    d. Arsenic
    (9-2–5)

24. Distinguish between local effect, remote effect, and cumulative effect as these terms relate to the action of poisons. (10-2)

25. Distinguish between *food intoxication* and *food infection.* (10-14)

ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. *a.* Maintenance of acid-base and electrolyte balance.
   b. Excretion of waste products of metabolism.
   c. Maintenance of osmotic equilibrium.
   d. Excretion of foreign substances such as dyes.

2. Most of the selective reabsorption occurs in the kidney tubules.

3. *a.* The rate of renal blood flow.
   b. The activity of the kidney tubules.
   c. The rate and efficiency of glomerular filtration.

4. *a.* Clearance tests.
   b. Excretion tests.
   c. Concentration-dilution tests.
   d. Retention tests.

5. Urease hydrolyzes urea plus water into ammonium carbonate.

6. SPS is a color stabilizer.

7. *a.* Ammonia, NH_3.
   b. Turbidity is most often due to the formation of mercury salts from improperly prepared Nessler's reagent or contamination.

8. A somogyi (zinc hydroxide) filtrate is considered the filtrate of choice since it does not yield substances which produce interfering colors upon nesslerization.

9. *a.* Occurring as the end product of purine metabolism, uric acid is a component of the total nonprotein nitrogen of the body.
   b. Elevated levels are due to renal disorders, blood dyscrasis, lead poisoning, liver disease, or gout.

10. *a.* Hexavalent phosphotungstic acid is reduced by uric acid to a lower valence in alkaline solution with the formation of a blue color.
    b. 4 to 6 mg-%.
    c. No particular significance.

11. Creatinine is one of the nonprotein nitrogens containing constituents of blood and has the formula $C_4H_7ON_3$.

12. *a.* Creatine is the anhydride of creatinine.
    b. First the creatinine is assayed and then the creatine is hydrolyzed to creatinine. Creatinine is assayed again and the difference after correction for differences in molecular weight represents the creatine level.

13. Because PSP dye is supplied in vials of 1 ml. each, the exact amount injected (some vials may contain slightly more to allow for loss in drawing the dye into a syringe).

14. Clearance tests measure the efficiency with which certain substances are removed from the blood by the kidney.

15. *a.* % clearance $= \frac{U}{B} \times 100$

$$\% = \frac{50}{100} \times 100$$

$$\% = 50\%$$

b. The volume of urine is considered in the performance of this procedure by referring to table 1. Hence, it does not enter into the calculations.

16. $C_m = \frac{UV}{B} \times K$

$U = 90$ mg./ml.
$V = 3.3$ ml./min.
Height $= (3 \times 12) + 9$, or 45 inches = 115 cm.
$K = 2.04$ (table 2)
$B = 10$ mg./ml.

$$C_m = \frac{90 \times 3.3}{10} \times 2.04$$

$C_m = 60.6$
This result is normal.

17. $C_m = \frac{UV}{B} \times K$

$U = 2.5$ mg./ml.
$V = \frac{75}{30}$ or 2.5 ml./min.
$B = 0.08$ mg./ml.
$K = 1.01$ (5′9″ = 69″ = 175 cm.
refer to table 2)

$$C_m = \frac{2.5 \times 2.5}{.08} \times 1.01$$

$C_m = 78$ ml./min.
This is normal.

18. $U = 1.6$ mg./ml.
$V = \frac{50}{30} = 1.6$ ml./min.
$B = 0.4$ mg./ml.
$K = 1$ (no correction needed)

$$C_s = U\frac{\sqrt{V\,K}}{B}$$

$$C_s = 1.6\frac{\sqrt{1.6\,(1)}}{.4} = 4\,(1.25) = 5 \text{ ml./min.}$$

This is not normal.

19. *a.* V = 0.33, therefore, use factor 0.35.
    U = 6 mg./ml.
    B = 0.2 mg./ml.

$$\text{Minimal clearance} = \frac{U}{B} \times 0.35$$

$$\text{Minimal clearance} = \frac{6}{.2} \times 0.35 = 10.5 \text{ or } 10 \text{ ml./ min. (rounded)}$$

b. No.
c. This result is probably not highly significant because of the low urine output.

20. *a.* There should be at least 7 points difference between the lowest and highest specific gravity. The volume of the specimen collected from 2000 hours to 0800 hours is usually at least 600 ml., but less that 800 ml. In kidney disease the volume of the night specimen is usually greater than 600 ml. and the specific gravity at least 1.020.
b. The specific gravity should be 1.003 or less in at least one specimen and the total volume of urine voided should be over 1200 ml.

CHAPTER 2

1. *a.* Rhythmic action of stomach.
   b. Openings in gastric mucosa.
   c. Dome at top of the stomach.
   d. Tissue beneath gastric glands.
   e. Valve into small intestine.
   f. Cells which secrete HCl.
   g. Column-shaped epithelial cells which line the stomach.
   h. Enzymatic self-destruction.
   i. Cells of the stomach which secrete an enzyme.
   j. Folds in the stomach.

2. HCl is formed in the parietal cells of tubular glands from plasma chloride ions and hydrogen ions from the dissociation of H_2CO_3. The cells secrete the acid through gastric pits in the mucosa.

3. 0.9 to 1.5.

4. *a.* Nerve to the stomach.
   b. Enzyme which acts on fats.
   c. Decreased gastric acidity.
   d. Surgical removal of the stomach.
   e. An organic acid which is the source of hydrogen for HCl.
   f. An enzyme which acts on casein.
   g. Increased gastric acidity.
   h. Absence of HCl.
   i. Enzyme which acts on proteoses.

5. *a.* A degree is defined as the amount of acid in 100 ml. of gastric juice which is neutralized by 1 ml. of 0.1*N* NaOH.
   b. A degree and a mEq/L are numerically equivalent.
   c. 15° to 45°.

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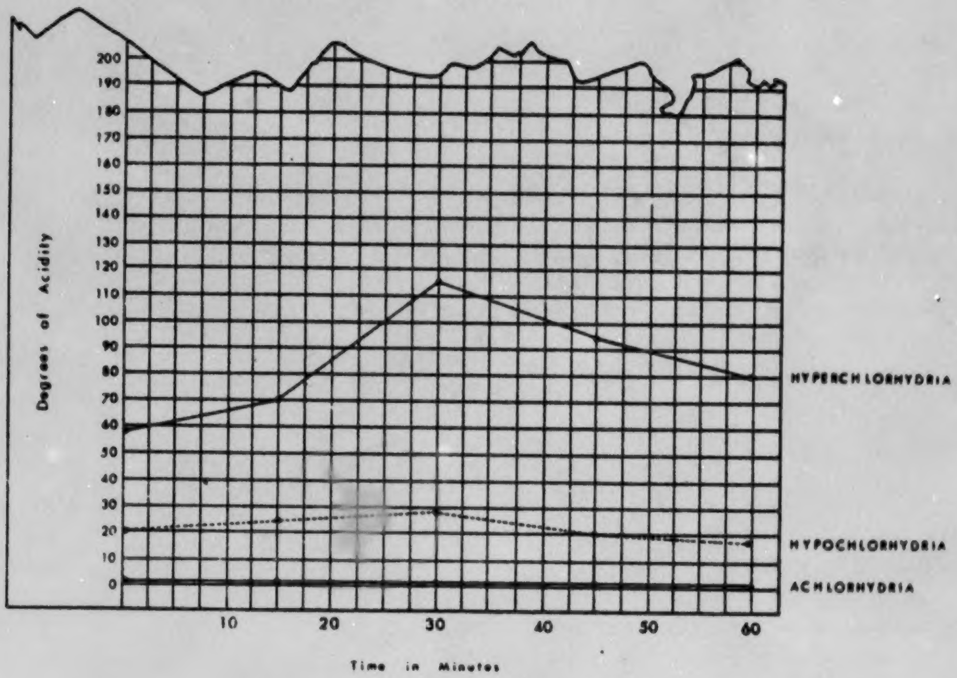
6.



CRE figure 1. Answer to Review Exercise No. 6.

7. Slightly grey to pale yellow.

8. Blood which is not fresh appears dark brown (coffee color) while fresh blood is bright red. The accumulated blood may also be detected chemically by the guaiac or benzidine test.

9. Titrate 5 ml. of gastric specimen (or some other measured volume) to the pink-red endpoint with phenolphthalein indicator. Calculate as follows:

$$\frac{\text{ml. of } 0.1N \text{ NaOH}}{\text{ml. of specimen}} \times 100 = \text{degrees combined HCl.}$$

10. To compute the degrees of free HCl, substitute in the formula as given in item 9 as follows:

$$\frac{0.5}{2.5} \times 100 = 20^\circ \text{ free HCl.}$$

11. Topfer's reagent (methyl yellow).

12. Combined gastric acidity is total HCl minus free HCl, or that portion which combined with organic substances.

13. *a.* Salmon pink.
    b. Pink-red.

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14. *a.* A dye, such as azure-A attached to an ion exchange resin, is given the patient orally. The amount of dye which appears in the urine is measured as an index of the amount of HCl which released dye in the stomach.
    b. This is generally accepted as a valid screening procedure for determining whether hypoacidity or hyperacidity exists.

CHAPTER 3

1. Electrophoresis is the migration of charged particles in an electrolytic solution resulting from an electric current flow.

2. A protein fraction in an electrophoretic system at its isoelectric point is electrically neutral and does not migrate.

3. The nature of the particle (size, charge) and the pH of the medium determine the rate and direction of migration of a particle in electrophoresis.

4. The serum protein fractions in order from anode (+) to cathode (−) are albumin, α_1, α_2, β, and γ globulin.

5. Liver diseases, myeloma, chronic infections, and sickle cell anemia.

6. Ferrohemoglobin solubility is a chemical test used to distinguish between hemoglobins "D" and "S" which migrate together electrophoretically.

7. Cellulose acetate has the advantages of increased resolution, greater speed of resolution, and the necessity for only micro quantities of specimen for electrophoresis.

8. Chromatography is the separation of mixtures by the use of their different absorptive properties in a common medium.

9. Serum (plasma) iron is the clinically significant iron, other than that normally present as hemoglobin, which is loosely bound to siderophilin for transport in the plasma.

10. Hot trichloroacetic acid simultaneously splits the iron from its carrier protein and precipitates the protein.

11. *a.* Separation of organic iodine or thyroxine.
    b. Digestion of organic iodine molecules to release inorganic iodine.
    c. Quantitation of inorganic iodine.

12. Ion exchange resins remove inorganic iodide.

13. The ceric-arsenious acid reaction involves reduction of yellow colored ceric ions to colorless cerous ion by arsenious acid through the catalytic effect of iodide ions.

14. *a.* Radiopaque dyes in X-ray.
    b. Iodinated amoebicides.
    c. Vaginal suppositories.
    d. Gram's iodine.
    e. Lugol's solution.
    f. Tincture of iodine.
    g. Merthiolate.
    h. Mercurochrome.
    i. "All weather" suntan lotion.
    j. Water in the analysis.
    k. Janitorial disinfectants.
    l. Nessler's reagent.
    m. Spilled mercury.

15. A hormone is a chemical substance produced by an endocrine gland.

16. Estrogens are usually assayed with vaginal cytologic examination by a physician.

17. Progesterone is produced in the corpus luteum of the ovaries and in the adrenal cortex.

18. Corticoids.

19. You must place 10 ml. HCl in the container before collection, label the container as to its content and the danger, and instruct the patient or ward personnel concerning the method of collection and danger of HCl.

20. *a.* Pituitary.
    b. Adrenals.
    c. Ovaries.
    d. Testes.
    e. Placenta.
    f. Also thyroid, islets of Langerhans (not mentioned in this section).

21. A *mouse unit* is defined as the least amount of estrus-producing gonadotrophin which induces desquamation of the vaginal epithelium in a spayed mouse.

22. 10 ml. of hydrochloric acid must be placed in the container before the specimen is collected to preserve the steroids and VMA.

23. Calculi analysis requires a number of reagents and manipulations which are time-consuming in a smaller laboratory. In addition, the incidence of calculi is not sufficient to warrant analysis in all laboratories.

CHAPTER 4

1. *a.* A clinical evaluation of an individual's state of intoxication constitutes a sobriety test. A blood alcohol determination is merely a laboratory procedure to determine the level of alcohol in the blood.
   b. UCMJ, Article 31.

2. No. Tinctures contain alcohol and this is a possible source of contamination.

3. A chain of custody receipt is a log showing by whom and how a specimen is handled from the time it is drawn until the test is run. It is important as evidence that a specimen was properly safeguarded, a factor to be considered if the report is used in a court of law.

4. Ethanol is transformed into acetaldehyde, CH_3CHO. Acetaldehyde is converted to acetic acid, CH_3COOH. Acetic acid is finally oxidized to carbon dioxide and water with the release of energy.

5. Prolonged use of alcohol results in liver damage, with all of the associated symptoms. Other effects include motor disturbances, vitamin deficiencies, and porphyuria. Lipuria may also result from alcoholism, and there are also toxic symptoms such as hypoxia. Hypoxia is characterized by lack of oxygen available to tissues.

6. *a.* Ethanol has the formula C_2H_5OH; methanol is CH_3OH. The former is far less toxic in moderate amounts, whereas methanol is a potent poison.
   b. 25 percent.

7. *a.* Hexavalent chromium of the dichromate is reduced by the alcohol to trivalent chromium, resulting in a change of color from yellow to green. The intensity of the color developed in the acid dichromate is proportional to the concentration of the alcohol.
   b. $Cr_2(SO_4)_3$.

8. *a.* Anstie's reagent is a potassium dichromate-sulfuric acid solution.
   b. It is used in the reduction procedure for the determination of blood alcohol levels.

9. Lactic acid and acetone.

10. Optical density varies somewhat with the temperature.

11. If tightly stoppered and refrigerated, the specimen may safely be considered stable for 5 days.

12. *a.* An antipyretic reduces temperature and an analgesic relieves pain.
    b. Salicylates act on the hypothalmus, an area of the brain which functions as a biological thermostat.

13. *a.* Aspirin is acetyl salicylic acid, a salicylate ester of acetic acid.
    b. Salicylic acid reacts with ferric nitrate in acid solution to form a red-purple iron complex.

14. *a.* Salicyates cause respiratory alkalosis from increased respiration following respiratory acidosis from increased CO_2 production. Metabolic acidosis results from impaired renal function and the loss of bicarbonate.
    b. (1) oxidative phosphorylation, (2) carbohydrate metabolism, (3) nitrogen balance, (4) fat metabolism, (5) enzymes.

15. This test will not detect the acetylated form of salicylic acid.

16. mg-% salicylic acid = mg-% sodium salicylate $\times \frac{138}{160}$ $40 \times \frac{138}{160}$ = 34.5 mg-% salicylic acid.

17. *a.* Carbon monoxide is a colorless, odorless, flammable gas, slightly lighter than air.
    b. Carbon monoxide has a greater affinity for hemoglobin than oxygen has. Consequently, the formation of carboxyhemoglobin results in anoxia or unavailability of oxygen to body tissues.

18. *a.* (1) concentration of carbon monoxide in the atmosphere. (2) time of exposure. (3) age and physical condition of the patient, especially his hemoglobin level.
    b. 0 to 5 percent.

19. *a.* Carbon monoxide, acid-liberated from a blood sample, reacts with palladium chloride in a microdiffusion cell, causing the release of metallic palladium which forms a "mirror" on the surface of the reagent.
    b. Oxyhemoglobin is reduced by sodium hydrosulfite.
    c. Formic acid and sulfuric acid.

20. The result should still be reliable if the test is properly performed.

21. It is sometimes necessary to prepare an acid distillate such as required for rapid spot tests. A portion of tissue (e.g., 100 g. of brain) is hemogenized in a cold Waring blender. The hemogenate is then transferred to a Florence flask together with a rinse of approximately 100 ml. of distilled water. To this mixture is added 225 g. of ammonium sulfate and 5 ml. concentrated sulfuric acid. The flask is connected to the distillation apparatus illustrated in figure 12.

22. *a.* Albumin.
    b. When it is heated the precipitate dissolves completely if it is due to alkaloids.

23. *a.* Alkaloids.
    b. Alkaloids.
    c. Halogenated hydrocarbons.
    d. Heavy metals.

24. *Local effect* means direct action on the part to which the poison is applied, such as corrosion and irritation; *remote effect* means the action of the poison on some organ remote from the seat of application or point of introduction. Sometimes, a poison shows no effect or only a slight one, until several doses have been taken. This is known as *cumulative effect.*

25. *Food intoxication* is due to a specific toxin produced outside the body such as that produced by microorganisms. *Food infection* refers to the presence of toxic-producing agents in the body and the consequent effects of their presence.

STOP– **1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.** **2. USE NUMBER 1 PENCIL.**

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VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed*. Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001) Activity of the kidney tubules can be measured by

    a. clearance and dilution tests.
    b. dye excretion tests.
    c. concentration and dilution tests.
    d. retention tests.

2. (001) The hydrolysis of urea results in the formation of

    a. ammonium hydroxide.
    b. ammonium carbonate.
    c. ammonium chloride.
    d. uric acid.

3. (001-002) The main difficulty in the hydrolysis of urea with Nessler's reagent is in

    a. color stability.
    b. urease instability.
    c. the formation of mold.
    d. deactivation of the enzymes.

4. (002) The normal range for a blood urea nitrogen in milligrams per 100 ml. of whole blood, serum, or plasma is

    a. 8 to 10.
    b. 10 to 18.
    c. 14 to 20.
    d. 18 to 26.

5. (002) Development of a color with the Berthelot reaction for the assay of ammonia depends upon the dissociation of

    a. ammonia.
    b. phenol.
    c. diacetyl monoxime.
    d. indophenol.

6. (002) Which of the following is found in uric acid?

    a. Protein.
    b. Sulfur.
    c. Ammonia.
    d. Nitrogen.

7. (003) Urine collected for a creatinine procedure must *not* contain

    a. thymol.
    b. toluene.
    c. acids.
    d. any of the above.

8. (004) What percent of PSP dye do normal kidneys excrete in the first 15 minutes after injection?

    a. At least 10 percent.
    b. Less than 25 percent.
    c. At least 25 percent.
    d. At least 65 percent.

9. (004) The *efficiency* of kidney function is measured by the

    a. concentration test.
    b. clearance test.
    c. excretion test.
    d. retention test.

10. (005) The formula $\frac{U}{B} \times 100$ is used to calculate

a. maximum urea clearance.
b. standard urea clearance.
c. average normal urea clearance.
d. minimal urea clearance.

11. (005-007) The formula $\frac{U\sqrt{VK}}{B}$ where

$K = \frac{1.73}{\text{body surface in square meters}}$ is used to calculate

a. average normal urea clearance.
b. minimal urea clearance.
c. maximum urea clearance.
d. standard urea clearance.

12. (008) The clearance of creatinine from the blood is primarily an index of

a. tubular function.
b. glomerular filtration rate.
c. selective reabsorption.
d. liver function.

13. (008) A patient with adrenal insufficiency would probably have a urine output which is

a. slightly increased.
b. significantly increased.
c. normal.
d. decreased.

14. (010) A band of visceral muscle at the bottom of the esophagus constitutes the

a. cardiac sphincter.
b. pyloric sphincter.
c. fundus.
d. pylorus.

15. (011) The surgical removal of the stomach is known as a

a. gastrotomy.
b. gastrectomy.
c. gastricism.
d. cholecystectomy.

16. (012) A mucoprotein present in gastric juice which is essential for the prevention of anemia is called

a. mucin.
b. transferrin.
c. peptidase.
d. intrinsic factor.

17. (012) Most absorption of the products of digestion takes place in the

a. small intestine.
b. stomach.
c. large intestine.
d. colon.

18. (012) Total gastric acidity depends upon the presence of

a. free acid.
b. acid phosphates.
c. organic acids.
d. all of the above.

19. (012) One degree of HCl is numerically equivalent to

a. 0.1 mEq/L.	c. 1.0 mEq/L.
b. 1.0 mg-%.	d. 10 mEq/L.

20. (012) Which of the following is *not* used to stimulate gastric secretion?

a. Epinephrine.	c. Alcohol.
b. Insulin.	d. Caffeine.

21. (013) Which of the following gastric fluid characteristics would be considered abnormal?

a. Fecal odor.	c. Slightly grey color.
b. Pale yellow color.	d. Lack of particulate matter.

22. (013) The Topfer's reagent endpoint signal is a pH of approximately

a. 2.0.	c. 5.0.
b. 3.3.	d. 8.5.

23. (014) Some investigators have proposed abandoning the terms *total* and *free* acid and using instead

a. undissociated hydrogen ion concentration.
b. titratable acidity.
c. hydrogen ion concentration.
d. all of the above.

24. (015) The migration of charged particles in an electric field defines

a. electrophoresis.	c. iontophoresis.
b. chromatography.	d. nephelometry.

25. (016) Albumin, α_1, α_2, β, and γ globulin are electrophoretic fractions of

a. hemoglobin.	c. amino acid.
b. serum protein.	d. serum lipoprotein.

26. (016) The medium rapidly gaining popularity because of its advantages for routine electrophoresis is

a. agar-gel.	c. starch-gel.
b. paper.	d. cellulose acetate.

27. (019-020) The physical property of absorption is used to separate mixtures in

a. chromatography.	c. electrophoresis.
b. electrolysis.	d. plasmaphoresis.

28. (020) The total iron binding capacity (TIBC) of serum is

a. UIBC + LIBC.
b. UIBC - LIBC.
c. serum iron + UIBC.
d. serum iron + UIBC.

29. (022) In PBI analysis, the time-reaction problem, speed of analysis, tedium of repetitive analyses, and capability for more analyses is effectively solved by

a. acid digestion.
b. wet ashing.
c. dry ashing.
d. automation.

30. (022) A chemical substance produced by the endocrine gland is called

a. a hormone.
b. a tyrosine.
c. an enzyme.
d. a catalyst.

31. (023) Pregnanediol and pregnanetriol are reduction products of

a. testosterone.
b. estrogen.
c. progesterone.
d. androgen.

32. (023) Androgen are assayed clinically by

a. the 17-ketosteroid procedure.
b. measuring testosterone.
c. the androsterone procedure.
d. precipitating digitonin.

33. (024-025) For the assay of 17-ketosteroids, reference laboratories require a urine specimen which is a

a. random specimen.
b. 12-hour specimen.
c. 24-hour specimen.
d. 250-ml. aliquote/24-hour specimen.

34. (025-026) It is preferable to measure VMA rather than catecholamines because of its

a. simplicity.
b. concentration.
c. specificity.
d. normal range.

35. (026) Which of the following urinary calculi are *most* commonly found in elderly Americans?

a. Magnesium ammonium phosphate.
b. uric acid.
c. calcium oxalate.
d. cholesterol.

36. (027) Which of the following could be found in the chloroform soluble portion of kidney stone?

a. Uric acid.
b. Indigo.
c. Calcium carbonate.
d. Fibrin.

37. (027) The best routine method for analysis of calculi is

a. chemical.
b. infrared spectroscopy.
c. X-ray diffraction.
d. pulverization.

38. (028) Amphetamine, according to the Gradwohl classification of poisons, is in the

a. alkaline-ether extraction group.
b. acid-ether extraction group.
c. volatile poison group.
d. heavy metals group.

39. (029) The best guideline in resolving medical-legal difficulties associated with toxicology testing is to

a. perform your duties conscientiously and not be concerned with legal responsibilities.
b. follow local policy and when in doubt consult your supervisor and/or the Staff Judge Advocate.
c. perform only those tests which the patient approves.
d. follow the physician's written request to the letter.

40. (029) Which of the following may be used to prepare the arm prior to venipuncture for a blood alcohol test?

a. Aqueous iodine.
b. Tincture of iodine.
c. Acetone.
d. Tincture of merthiolate.

41. (029) The purpose of a *chain of custody receipt* in handling a blood specimen is to assure

a. technical accuracy.
b. reproducibility.
c. security.
d. a lack of criticism.

42. (030) Ethyl alcohol is the same as

a. CH_3COCH_3.
b. ethanol.
c. aminoethane.
d. antabuse.

43. (030) Most of the alcohol in the blood is

a. rapidly excreted in the urine.
b. exhaled from the lungs.
c. reabsorbed in the kidney tubules.
d. excreted in perspiration.

44. (031) In the dichromate procedure for alcohol,

a. trivalent chrominum is reduced.
b. alcohol oxidizes hexavalent chromium.
c. Anstie's reagent is oxidized to a green color.
d. hexavalent chromium is reduced by alcohol to trivalent chromium.

45. (031) Before a blood specimen is reported as positive for alcohol, the patient's plasma should be checked for acetone to rule out

a. lipuria.
b. ketonemia.
c. Ketonuria.
d. methanol.

46. (031-032) An antipyretic commonly used therapeutically is

a. methyl salicylate.
b. disulfiram.
c. acetyl salicylate.
d. lithium oxalate.

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47. (032) A blood salicylate level of 70 mg-% is considered

a. subclinical.
b. therapeutic.
c. toxic.
d. too low to measure.

48. (033) Pure carbon monoxide gas is

a. odorless.
b. pungent.
c. acrid.
d. sulfurous.

49. (034) The most reliable method of determining the extent of carbon monoxide in blood makes use of

a. sodium hydroxide.
b. palladium.
c. electrophoresis.
d. chromatography.

50. (034) In screening a toxicological specimen for volatile reducing substances, Anstie's reagent reacts with

a. cynogen chloride and ethanol.
b. cyanides, acetone, and methanol.
c. ethanol, methanol, and acetone.
d. sulfides, acetaldehyde, and ethanol.

51. (034-036) Which of the following are *not* considered volatile poisons?

a. Phenols.
b. Cyanides.
c. Sulfides.
d. Barbiturates.

52. (035) A chemical which may be detected from an acid distillate by the formation of insoluble tribromphenol is

a. phenol.
b. caffeine.
c. cyanide.
d. trichloroethylene.

53. (036) If you were to sustain hand burns with sulfuric acid, you would be suffering from an effect classified as

a. cumulative.
b. remote.
c. local.
d. systemic.

54. (037-038) The susceptibility of children to poisons in comparison to adults is

a. variable.
b. less.
c. the same.
d. greater.

55. (038) An example of chronic poisoning is likely to be found in cases of

a. suicides.
b. food handling.
c. fuel handling.
d. overdosage.

56. (038) LSD may be classified toxicologically as

a. a corrosive.
b. an irritant.
c. neurotic.
d. a volatile poison.

57. (038) Food poisoning due to a specific toxin produced outside the body is best described as

a. enteritis.
b. food intoxication.
c. food infection.
d. an infusion.

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CDC 90411

MEDICAL LABORATORY TECHNICIAN - CLINICAL CHEMISTRY AND URINALYSIS

(AFSC 90470)

Volume 4

Laboratory Procedures in Urinalysis

Extension Course Institute

Air University

Preface

THIS FINAL volume of CDC 90411 discusses concepts in urinalysis at the 7 level. Chapter 1 begins with a review of proper microscope adjustment and the rather complex area of critical illumination. Other general considerations such as specimen collection and preservation are also reviewed in Chapter 1.

In Chapter 2, we will turn our attention to renal anatomy and physiology which will help us better understand results achieved in the laboratory as discussed in Chapters 3, 4, and 5.

Chapter 3 is a fairly basic presentation of the physical characteristics of urine and their meaning. The two most difficult and meaningful areas of urinalysis are treated in Chapter 4, Microscopic Examination, and in Chapter 5, Chemical Examination. In these last two chapters, some of the practical aspects of urinalysis are presented in depth sufficient to challenge most students. For example, in Chapter 5, you will discover that simple qualitative tests for urinary constituents can be very misleading in certain cases. In studying Volume 4, you should realize that urinalysis is an extremely valuable diagnostic discipline as well as a complicated one. It is not a subject to be dismissed lightly.

Foldout 1 is included at the back of the volume.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to TECH TNG CEN (MSSTW), Sheppard AFB, Texas 76311.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Study Reference Guides, Chapter Review Exercises, Volume Review Exercise, and Course Examination) consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFB, Alabama 36114, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 36 hours (12 points).

Material in this volume is technically accurate, adequate, and current as of February 1969.

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General Considerations

WE ARE CONSTANTLY reminded that our purpose within this career field is to support patient care. It is difficult to imagine a single category of laboratory tests which relates more directly and frequently to patient care than an analysis of one sort or another on urine. It is probably safe to say that a routine urinalysis is the most frequently performed laboratory test.

2. In beginning this brief introspection into the study of urinalysis, let us observe the danger signs that confront us. First, urinalysis is *not* a simple and dull subject, though it is often considered as such. Second, it is *not* a monotonous chore which, at best, does the patient no harm. Finally, the urinalysis section of a laboratory *is* a vital and vibrant area of direct concern to the patient and to the physician. If you doubt this, just ask any practicing physician how he evaluates the importance of this examination. The number of urine tests you are likely to perform in your career will far exceed the number of more complicated and involved determinations of other kinds.

1. Review of Microscopy

1-1. It is impossible to perform an accurate urine microscopic examination without an adequate optical and mechanical system. In this section we will discuss proper use and care of the microscope at the 90470 level. The understanding of an acceptable method of adjustment and illumination is more rewarding than trial and error technique.

1-2. **Preliminary Adjustment of the Microscope.** The modern microscope is a precision optical instrument which, like all fine instruments, requires care in its use. It also requires knowledge of the instrument on the part of the user. The relationship of the microscope lamp to the microscope is critical, because this relationship governs the intensity of the illumination which is available, as well as the quality of the final image which the eye sees. Since microscopes are seldom in constant use, it is desirable to maintain the relative position of lamp to microscope by means of a fixed mounting. This avoids the necessity of reestablishing the lamp-microscope relationship each time the scope is used. A microscope mounting board may be fabricated locally.

1-3. Preparatory to setting up the illumination system, review the components of the microscope illustrated in figure 1. After placing a slide on the microscope stage, swing the 10X objective into place. Adjust the microscope mirror so that the slide is illuminated. Move the condenser to its uppermost position (see substage control knob, fig. 1). Using the coarse focus adjustment of the microscope, obtain an image of the specimen on the slide. Next, turn the ocular focusing control to zero. Adjust the interpupillary distance control so that the lateral spread of the two eyepieces is comfortable to the eyes and only one image is seen. While doing this you will see that the image of each eyepiece fuses, to appear as a single circular image. Note for future reference the scale setting which is on the front of the microscope between the eye tubes.

1-4. With the objective fine focus adjustment, obtain a critical focus of the specimen on the slide, looking only through the eyepiece which does not have the ocular focusing control. For convenience, a piece of opaque paper may be placed over the eyepiece of the tube with the ocular focusing control. After obtaining a critical focus, switch the paper to the nonfocusing control eyepiece so that the eyepiece is obstructed. Do not further touch the objective fine focus adjustment. Using the knurled ring of the ocular focusing control, bring the image into fine focus for your eye. The microscope will then be adjusted to accommodate for small differences in refraction of your two eyes. At this point note the numerical setting on the ocular focusing control ring for future reference. A person having astigmatism necessitating constant use of his glasses may wish to use a special set of oculars designed for use with spectacles; however, these are not available through regular supply channels in the Air Force. The proper name for the system of illumination which will now be described is "Kohler of illumination."

1-5. *Focusing the lamp filament.* Before continuing, study the components labeled in figure 2. These will be referred to in the description which follows. Place a piece of filter paper or a white 3″ x 5″ card over the mirror of the microscope as shown in figure 2. Be sure that the flat side of the mirror is facing forward. (The curved side of the mirror is never to be used with an artificial light source.) Remove the filter holder from the lamp. Close the iris diaphragm of the lamp as far as it will go. Do not force this or any other control. Align the lamp so that it is directly in front of the microscope and is at a distance of approximately 8 to 12 inches from the microscope. By means of the knurled knob at the back of the lamp base, shown in figure 2, adjust the vertical position of the lamp so that the light falls on the card or filter paper covering of the microscope mirror. With the filament focus control, move the lamp tube back and forth until the filaments of the lamp bulb are sharply focused on the card or filter paper shown in figure 2. Adjust the horizontal and vertical directions of the lamp so that the image of the filaments is centered on the mirror.

1-6. Focusing the lamp filaments on the mirror is a convenient method of establishing the illumination. However, an even more accurate method is to take a second flat mirror and po-

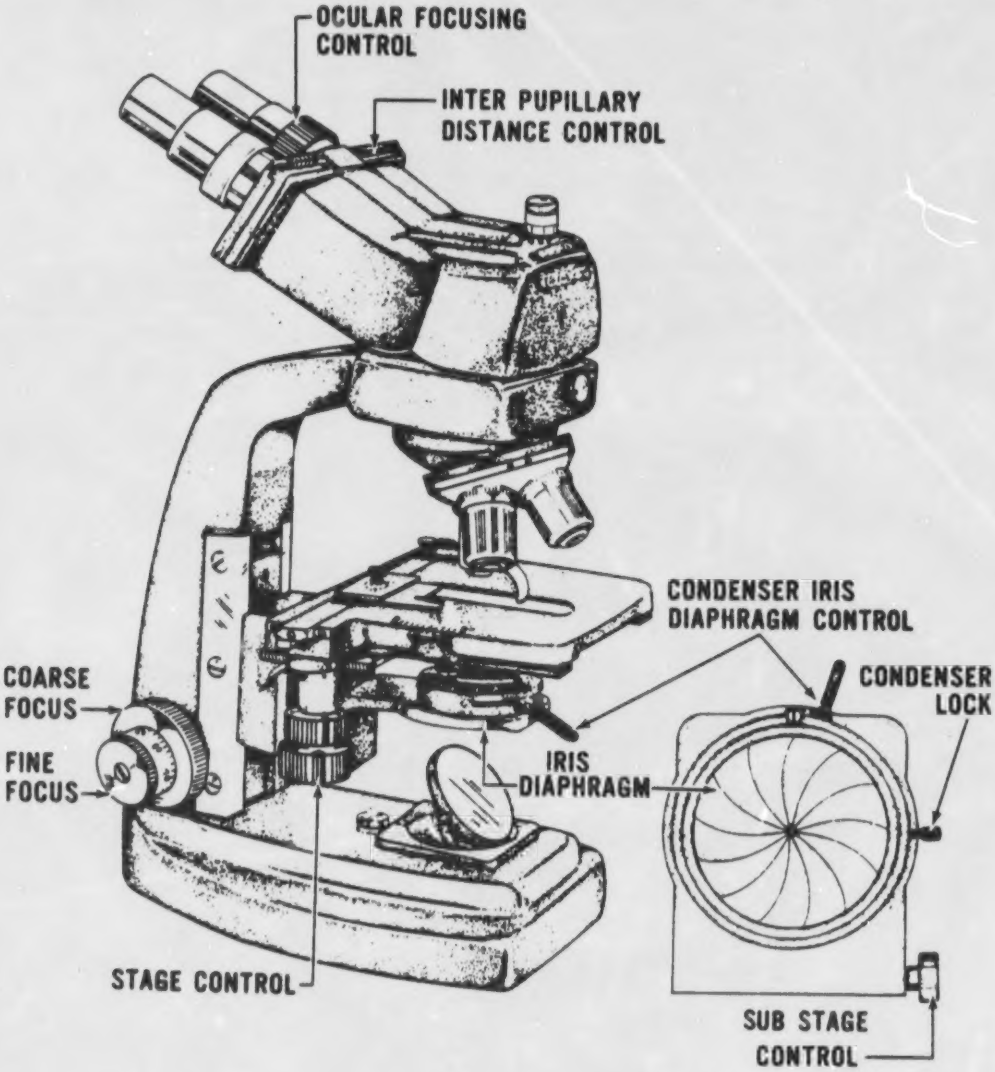

OCULAR FOCUSING CONTROL
INTER PUPILLARY DISTANCE CONTROL
CONDENSER IRIS DIAPHRAGM CONTROL
CONDENSER LOCK
COARSE FOCUS
FINE FOCUS
IRIS DIAPHRAGM
STAGE CONTROL
SUB STAGE CONTROL

Figure 1. Clinical laboratory microscope.

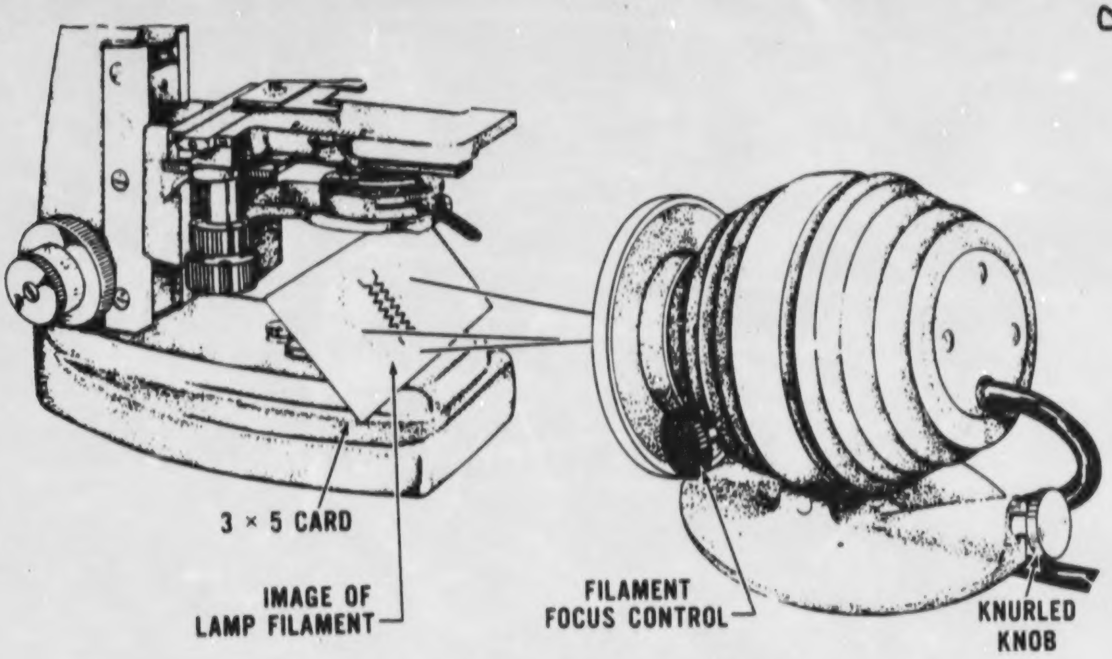


Figure 2. Microscope lamp illumination.

sition it adjacent to the microscope mirror so that one can see the underside of the condenser iris diaphragm. The condenser iris diaphragm is closed so that its opening is but a pinpoint. Adjust the lamp filament focus controls so that the image of the lamp filaments is in focus and centered on the underside of the condenser iris diaphragm.

1-7. *Focusing the condenser*. Check the microscope image of the slide object. Be sure this is still in focus. Now be sure that the condenser iris diaphragm is closed. With both the condenser iris diaphragm and the lamp iris diaphragm closed, move the microscope condenser up and down until a sharply defined image of the lamp iris diaphragm is obtained in the field of view. Once again the image of the object on the slide should be in sharp focus. Adjust the microcope mirror so that the image of the lamp iris diaphragm is centered in the middle of the field of vision. Open the iris diaphragm of the lamp until the full field of vision seen through the microscope eyepieces is fully illuminated. No further adjustment to the lamp or microscope condenser is required.

1-8. Some microscopes may require an additional auxiliary lens, which is of the swing-out variety, beneath the condenser before full field illumination is obtained. This depends on the condenser attached to the microscope. In such instances the auxiliary lens is kept out of the optical pathway until the lamp adjustments and condenser adjustments are completed.

1-9. Replace the filter holder on the lamp, being careful not to disturb the position or adjustment of the lamp. You will note that the filter holder has a blue-tinted color correction filter, a white diffusion disc, and a snapring to hold the tube components, color filter, and diffusion disc in place. There is room for additional filters in the external rack of the filter holder.

1-10. Once the illuminating lamp and the microscope condenser have been focused, the only other adjustment which must be made is that for the condenser iris diaphragm. This iris diaphragm is not intended to control the level of illumination seen in the microscope. Instead, it is intended to serve as a part of the optical system which is responsible for resolution of the image. There is only one proper iris diaphragm control setting for each of the objectives on the microscope. In order to properly adjust the iris diaphragm, carry out the steps outlined in the following paragraphs.

1-11. *Adjusting the condenser iris diaphragm.* The condenser iris diaphragm should be opened fully. One of the oculars is removed (eyepiece of the microscope), as shown in figure 3,A. If you look down the eye tube you will see that the back lens of the objective is fully filled with light. At this point if you close the iris diaphragm of the condenser to its fullest closed position, you will see that there is but a pinpoint of light visible

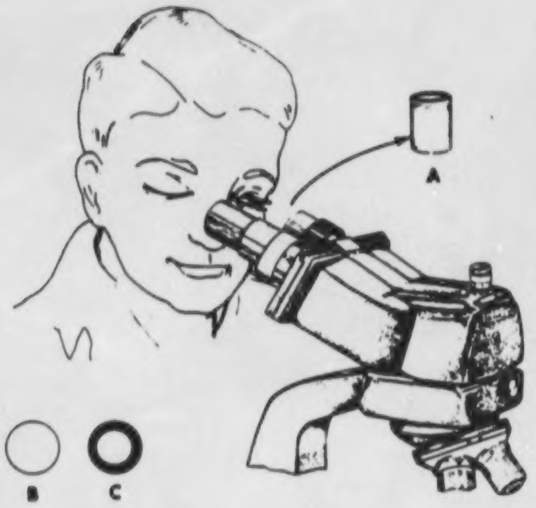


Figure 3. Adjusting the condenser diaphragm.

at the back lens of the objective. Once again the iris diaphragm of the condenser is fully opened (fig. 3,B) and slowly closed until the diameter of the back lens' illumination is being slowly reduced. Continue to close the iris until approximately two-thirds to three-quarters of the diameter of the back lens is now fully illuminated. This leaves the outermost one-third to one-fourth of the back lens of the objective nonilluminated as shown in figure 3,C. Replace the ocular (eyepiece). If you follow the preceding steps, your microscope will be properly adjusted for Kohler illumination which will produce the finest resolution the microscope is capable of offering.

1-12. When your microscope has a revolving nosepiece and you are using more than one objective, you will find that it becomes necessary to slightly readjust the entire series of adjustments which we have previously described. For this reason it is advisable to make your first alignment using the 10X objective. Then go to your oil immersion objective and make the fine adjustments necessary to produce a sharply focused centered image of the iris diaphragm of the lamp. Also, properly adjust the iris diaphragm of the condenser. With the lamp focus, and mirror condenser adjustments aligned for the oil immersion lens, we do not further adjust the lamp or condenser for the objectives except for the condenser iris diaphragm. Each objective will require a slightly different setting of the condenser iris diaphragm to produce the desired reduction in illumination of the back lens of the objective.

1-13. In a similar manner, the extent to which the lamp iris diaphragm must be opened to produce a fully illuminated field will vary according to the objective used. For convenience, allow this to remain at the setting required for the lowest power objective being used (usually 10X).

1-14. Never attempt to use objectives having a high numerical aperture (N.A.) value with a condenser having a lower value. Ask your pathologist, laboratory officer, or supervisor to check your microscope and its optics in this regard. However, this loss in resolution is relatively slight in comparison with that obtained when the illumination is improperly designed for the oil immersion objective.

1-15. The intensity of illumination should be controlled with neutral density filters. Do not attempt to control the intensity of light as your eyes see it by opening or by using the condenser iris. This is incorrect. Likewise, you cannot control the intensity of illumination with the lamp iris. The lamp iris is used only to cut out extraneous light once it has been opened sufficiently to produce a full field of illumination. The intensity of illumination may be controlled by regulating the lamp voltage, in which case suitable color correction filters to produce a white or blue-white light are required. If the voltage cannot be controlled we must use neutral density filters to reduce the amount of light which we receive. Within limits, the intensity of illumination may also be controlled by placing the illuminating lamp closer to, or further from, the microscope. However, this will require readjustment of the lmap focus. Once you have properly established the relationship of the microscope lamp to the microscope and adjusted the lamp and condenser focus, then these relationships can be maintained by locking the lamp to a lamp board if your scope is mounted as suggested.

1-16. Before concluding our discussion of the microscope, let us briefly review some points of good technique which should already be thoroughly familiar to you. A monocular microscope should be used with both eyes open. Lenses must not be touched except with lens paper. Mirrors, slides, cover glasses, and other microscope equipment may be cleaned with lint-free tissue or with a soft cloth. Do not insert or remove a slide under high power. Never allow an objective to touch a cover glass. Always locate the object under low power before trying to find it under high power. Most microscopes are parfocal, which means the viewer can switch from one power to another with minimal refocusing. Oil is always wiped from the oil immersion objective when you are finished using the microscope. Switch to low power when finished, rather than leaving the oil immersion objective in a viewing position. Last of all, keep the microscope covered when it is not in use.

2. Collecting and Preserving Specimens

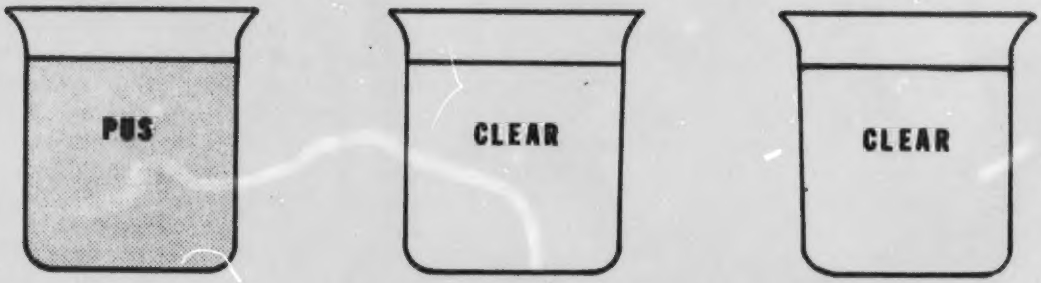
2-1. As simple as it is to obtain a urine specimen, reliability of results depends upon reasonable care and precautions associated with initial processing in the laboratory. Few technicians would treat "more valuable" specimens like cerebrospinal fluid as lightly as urine specimens. Quantitative determinations on urine are of clinical value only when performed on properly collected and prepared 24-hour specimens. The accurate collection of such specimens from ward patients and, most particularly, from outpatients has for many years presented certain technical and esthetic problems. Ward personnel are extremely reluctant to store urine specimens during the collection period in refrigerators containing drugs or foods. This is equally true of specimens collected in the home. However, the bottle must be kept in the refrigerator for certain types of urine chemical analyses. With some care, one can assure that the outside of the bottle is clean and free from urine, which might contaminate other contents of the refrigerator. If a preservative or other additive has been placed in the bottle, it is essential that the contents be gently but thoroughly mixed after each addition. After collection is completed, the specimen should be brought to the laboratory immediately. Let us consider some of the common areas of concern dealing primarily with handling of the specimens.

2-2. **Collecting the Specimen.** How a specimen is to be collected is determined by the physician with technical assistance from laboratory personnel. However, keep in mind that the responsibility for the accuracy of urine collections lies first with laboratory personnel, who must be familiar with the necessary preservatives or other additives required, and who supply properly prepared collection bottles to the wards or directly to outpatients. The second responsibility is that of physicians and nurses, to explain to the patient the proper method of collection, as well as the need for complete collections. The third responsibility is that of the patients. Without this, accurate collections are impossible. This is one of the transitional areas in which neither the clinician nor the laboratory always knows precisely how each specimen was collected.

2-3. The nursing service of a hospital can often be of tremendous value in assuring that a specimen is of the type the physician intended and what the laboratory thinks it is. To cite a few examples, an early morning specimen differs in clinical significance from one collected later. One cannot always assume that a sample which arrives in the laboratory in the morning was actually collected in the morning. It is also of concern that specimens be fresh or properly preserved. Hyaline casts, especially, will dissolve in neutral urine of low specific gravity or in any urine that is alkaline. Urine which has been standing becomes alkaline due to bacterial and chemical activity. In many cases, even if laboratory examination of the specimen is prompt, the urine may have been unsatisfactory when it arrived. It is your responsibility as a technician to evaluate specimens for technical acceptability.

2-4. Although a request form of the 514 series has a block for date and time of collection, the entry one finds rarely has any relationship to the time the patient voided. This is not so much a case of negligence as a practical problem. For outpatients, the requirement almost necessitates an entry by the patient himself, and this is more than the most optimistic technician can consistently expect. An even greater error is introduced by incomplete specimens. Not infrequently, the patient is improperly instructed with regard to the necessity of collecting *all* voidings within the 24-hour period. The patient often forgets to void into the container, is too preoccupied to obtain his or her container from the storage refrigerator, or else is too shy to bring his specimen to the collection bottle. Because of these personal problems and environmental objections, such as may be encountered in a school, office, or on the flight line, a mutually satisfactory time usually can be determined. You, as the technician, should decide from the physician's request, the situation of the patient and laboratory scheduling the optimum time for the collection. If the physician requests a specific period of time for collection, of course, the orders should be followed to the letter. Parents may require special assistance in securing urine specimens from small children. You may provide plastic collecting containers designed for pediatric patients and give instructions in container use.

2-5. In reality, the best practice is for collection to be made in or near the laboratory. For inpatients, there are also problem areas. Specimens transferred from other receptacles may be contaminated. But perhaps more common, specimens sometimes remain on the ward until personnel are free to pick them up or send them to the laboratory. Laboratory slips are frequently completed ahead of time; therefore, as with outpatients, these forms do not always reflect accurate entries regarding time of collection. This does not imply a criticism of the nursing serv-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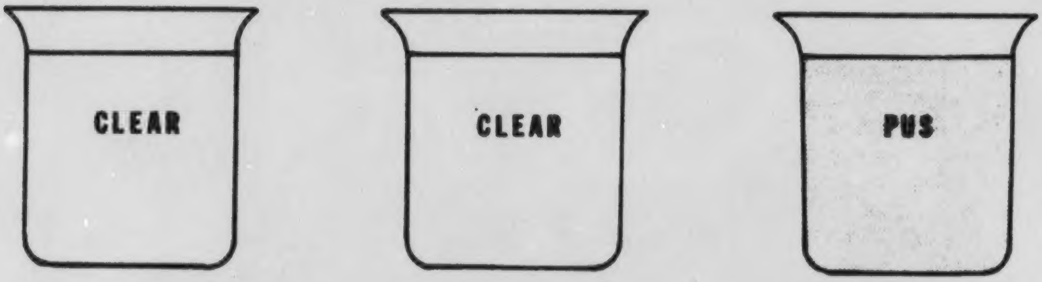
ONLY 1ST GLASS CLOUDY MAY INDICATE URETHRITIS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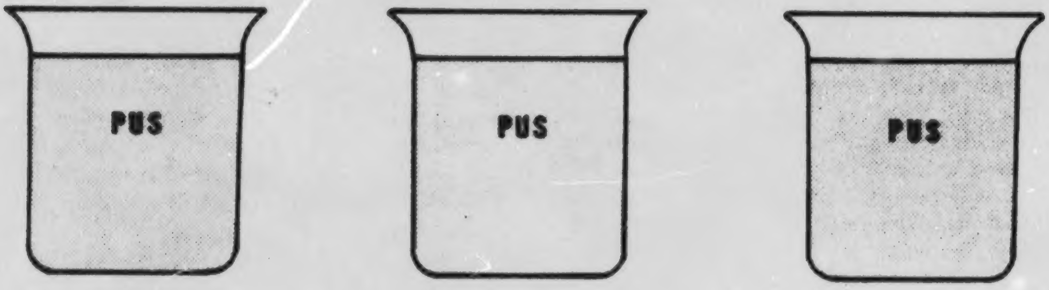
ONLY 3RD GLASS CLOUDY — PROSTATITIS OFTEN CAUSES THIS



ALL THREE GLASSES CLOUDY — BLADDER AND/OR RENAL INFECTION SUGGESTED

Figure 4. Observation of the three-glass urine specimen test.

ices. It is merely a fact which must be dealt with if the result is to be meaningful.

2-6. Collecting a specimen sometimes requires special instructions. It is assumed you are thoroughly familiar with the terms *random specimen, clean catch, midstream, two-glass,* and *three-glass.* Figure 4 indicates how laboratory observation of three-glass urine specimens could be interpreted by the physician. Needless to say, the diagnosis requires more than laboratory observation, but this does illustrate the value of three-glass specimens.

2-7. In advising outpatients on collection techniques, there is a tendency for the technician to assume too much or in some cases to be purposefully vague, perhaps to avoid embarrassment. A patient may not have the slightest idea what a midstream specimen is or how to collect it. This also applies to other special instructions. Instructions should be given in an explicit and professionally tactful manner. Sometimes the special handling of a specimen involves more chance for error than a slightly more casual approach. Consider, for instance what is necessary to obtain a clean-catch specimen. A clean-catch procedure which requires elaborate instructions and the use of cleansing agents is less desirable than a simpler method. Commercial agents such as "phisohex" must be used carefully or the urine will become contaminated with the cleansing agent. Some of these are potent antibacterial agents and could render the urine sterile before it is cultured. The subject of urine cultures will be covered in greater detail in a volume of the microbiology course. However, it is mentioned here that a simple procedure is often better than an involved one by the very reason of its simplicity.

2-8. It is expected that all of the usual laboratory precautions will be observed, including the use of clean or sterile containers. Rinsing a bottle and using it over again and again is *not* good technique. At least thorough washing with detergent and hot water is required. Contaminants, and especially molds which adhere to the container, can confuse a microscopic analysis. It might be argued that bottles should be sterilized as a good hospital practice to avoid possible contamination to individuals. Urine bottles from a contagious case (e.g., hepatitis) must certainly be sterilized. Mere rinsing is a widespread practice but hardly one to be recommended. Ordinary detergents do *not* inactivate viruses or even eradicate bacteria. Finally, cracked or chipped bottles should be discarded. Saving faulty glassware is false economy and downright dangerous in any laboratory situation. The use of broken glassware is inexcusable! If you work in a laboratory, it is suggested that you discard all broken or chipped glassware which cannot be economically repaired. This does not apply just to urinalysis. Glass items which can be repaired must not be used in the interim. If possible, substitute plastic or other nonbreakable items as a matter of economy and laboratory safety.

2-9. **Preservation of Urine.** There is no substitute for a fresh urine specimen, and in all cases analysis should be accomplished as soon as possible. On occasion, however, it is not possible to analyze the sample immediately (e.g., 24-hour specimens). A delay in analysis leads to degeneration of the formed elements and decomposition of chemical constituents. An example is the breakdown of urea into ammonia by the action of contaminating microorganisms.

2-10. Deterioration may be inhibited by the use of some form of preservation. Always keep specimens refrigerated when they cannot be promptly analyzed. It is important that specimens warm to room temperature before analysis is performed to reduce turbidity and enable the technician to make accurate quantitative measurements, including specific gravity determinations. A number of chemical preservatives can be utilized with varying degrees of effectiveness. The following are recommended for use *in conjunction with refrigeration.*

a. Toluene (sufficient quantity added to form a thin layer over the specimen) is a very satisfactory inhibiting agent used when chemical analysis is required because the reagent does not interfere with most chemical tests. Anaerobic bacteria which might be present in the sample continue to multiply, however. It is necessary either to remove the toluene before measuring portions of the specimen or to pipette from below the surface. Toluene is not recommended for collection of urine specimens for steroid determinations. Free steroids are extracted into the toluene, while ketogenic steroids and other water soluble conjugates will remain in the urine. Unless care is taken to mix the specimen thoroughly, part of the free steroids will be lost and low values will be obtained.

b. Formalin (about 4 drops/100 ml. urine) is the best preservative for formed elements. It interferes with some chemical analyses, and should not be used when sugar concentration is to be determined by reduction methods. Formalin also inhibits the Obermayer test for indican.

c. Thymol may be added to the extent of a few small crystals for every 100 ml. of urine. It gives a false positive albumin test, however, and may interfere with tests for bile.

d. Special preservatives include hydrochloric acid for Vanilmandelic acid (VMA), serotonin, and other analyses as discussed in Volume 3 of this course.

2-11. If a chemical preservative has been added to a urine specimen, the type and amount must be indicated on the label of the collection bottle. Always follow instructions of the reference laboratory when you preserve specimens for shipment. The Handbook of Reference Material which was furnished for your use in this course contains more extensive information on methods of preserving urine for special tests conducted by reference laboratories.

2-12. In handling 24-hour specimens, the laboratory should insure that the entire specimen is well mixed. Measure the total volume in a 1- or 2-liter graduated cylinder, to the nearest 5 ml. Whenever possible, collection periods should *not* begin on Friday or Saturday, to avoid having to store specimens over the weekend. If this is unavoidable, store the entire specimen in the refrigerator or freezer until shipped.

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Renal Functions

ONE REASON THE clinical laboratory performs so many urinalysis procedures is because of the vital importance of the kidneys. There is an ever-increasing interest in the role of the kidneys in maintaining electrolyte and fluid balance and in their relationship to certain diseases. As one investigator of renal function observed, "The composition of the blood and the internal environment is determined not only by what the mouth ingests but by what the kidneys keep."[1] The kidneys maintain this internal environment by filtering blood, by selective reabsorption, and by the secretion of foreign substances from the blood.

2. A variety of urine tests have been established which aid the physician in diagnosing and treating many disorders. To understand the importance and meaning of urinalysis, you should have some knowledge of the kidney itself. In this chapter you will become familiar not only with the structure of the kidney and with the physiology of the normal kidney, but with the correlation between certain diseases and the functions of the kidney.

3. As you gain insight into the application and importance of urinalysis tests, you will be less inclined to draw conclusions about the clinical condition of the patient. If you appreciate the involved relationships which exist between a laboratory test and the diagnosis of a disease, you will perform the test with greater care and interest. You will realize that a "normal" urine result can be as valuable to the physician as an "abnormal" report. It is important that you know the clinical impact and importance of your work, and at the same time refrain from the role of a diagnostician. Always think in terms of your responsibilities as a clinical laboratory technician and the welfare of the patient.

3. Anatomy of the Kidney

3-1. If an engineer attempted to design a filtration system, his design could hardly be more successful than the kidney. The kidney is a remarkable organ, quite complicated in structure and function. As William Bowman, an English physiologist (1816–1892), stated in 1842, "It would indeed be difficult to conceive a disposition of parts more calculated to favor the escape of water from the body. . . ." Of course, the functions of the kidney are vastly more complicated than Bowman implied or realized. In this section you will learn the basic structures of the kidney by studying this "disposition of parts."

3-2. Nearly everyone is generally familiar with the appearance of a kidney. The kidneys are bean-shaped organs situated in the posterior part of the abdomen on either side of the vertebral column between the 12th thoracic and the 3rd lumbar vertebrae. Because of the position of the liver, the right kidney is slightly lower than the left. The kidneys are not rigidly attached but move slightly with respiration. Each kidney is enclosed by a thin, translucent capsule which is penetrated by a number of blood vessels. There is an indentation known as the hilum which expands into a renal sinus. The kidney consists of several more or less distinct lobes which become less apparent with age. If you slice a kidney open, you will observe the lobed appearance in the central portion or medulla. The peripheral portion of a kidney is known as the cortex and is shown in figure 5. The appearance of the cortex varies considerably with pathological conditions. Normally, the cortex of a kidney appears granular with ray-like structures known as medullary rays. These rays are visible in the cortex as illustrated in figure 5, and not in the medulla as the name would imply. The medullary ray is composed of branched, collecting tubules and limbs of the loop of Henle. The lower portion of the loop of Henle extends into the medulla.

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[1] Smith, H. W., *Lectures on the Kidney*, U. of Kansas, Lawrence, Kansas.

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3-3. Structures directly related to the kidney, but not a part of the kidney, include the extra renal blood vessels, ureters, bladder, and urethra. Blood is supplied to the kidneys by way of the abdominal aorta, and returns to the heart by way of the inferior vena cava. Two renal arteries lead from the abdominal aorta to supply blood to the kidneys. There are two ureters, one leading from each kidney to the bladder. They are usually 12 to 15 inches long and carry urine from the kidneys to the bladder with the aid of muscular action. From the bladder, urine is passed from the body through the urethra.

3-4. The functional unit of a kidney is the nephron, which can be seen in figure 6,A. The nephrons average 50 to 55 millimeters in length and are situated in the cortex close to the medulla. There are approximately 1,300,000 to 2,000,000 nephrons in each kidney. If all of the tubules of both kidneys were stretched out in one direction they would extend for approximately 75 miles! Blood is supplied to each

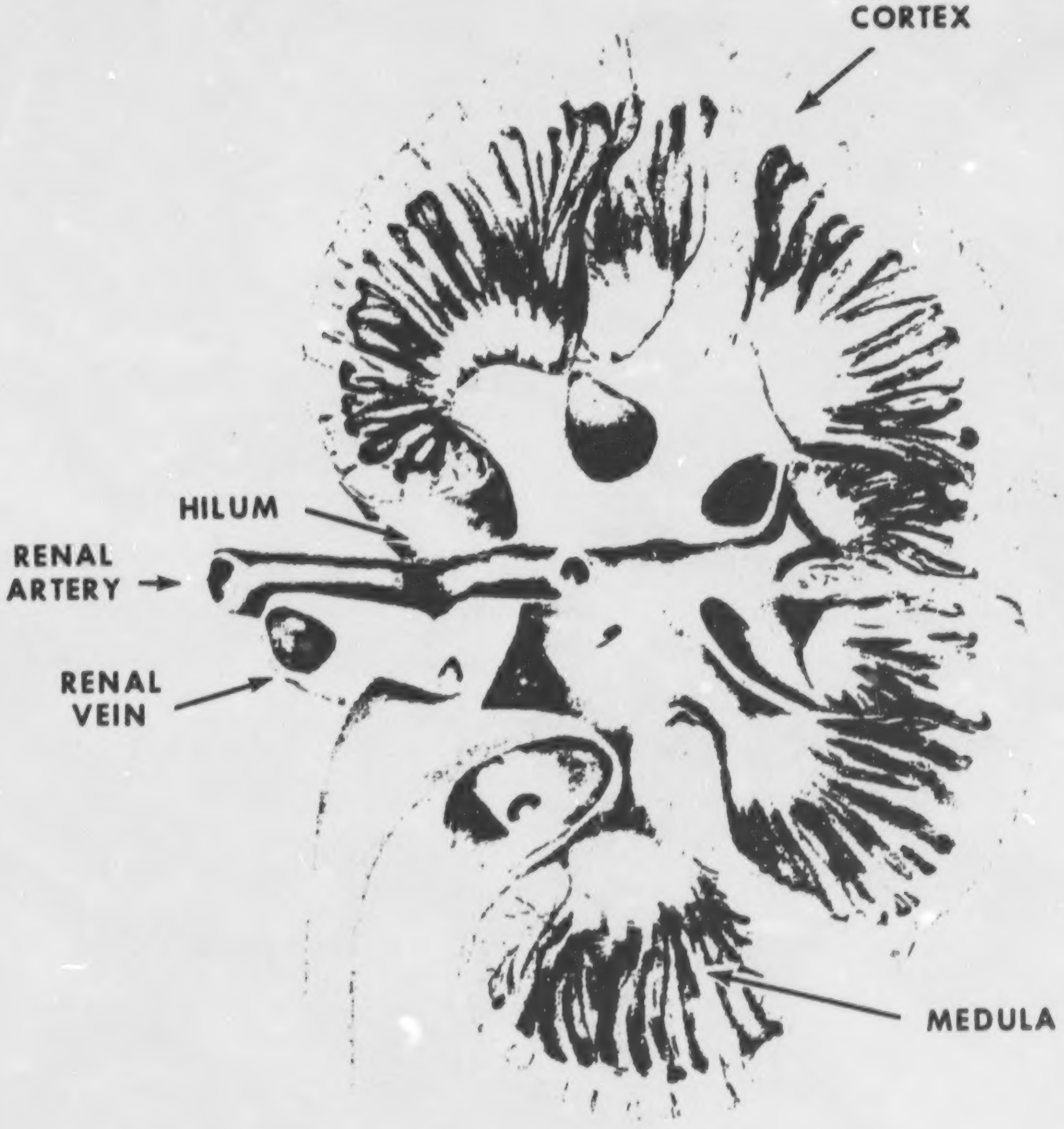

Figure 5. Cross section of the kidney.

nephron unit by means of an afferent arteriole leading from branches or endings of the two renal arteries. One afferent arteriole may supply more than one nephron. Blood is carried from the unit by means of an efferent arteriole through a capillary network into branches of the renal vein for return to the heart via the inferior vena cava. The nephron unit consists of four parts: the glomerulus; the proximal convoluted tubule; the loop of Henle; and the distal convoluted tubule. The glomerulus is also referred to as the renal corpuscle. A glomerulus averages from

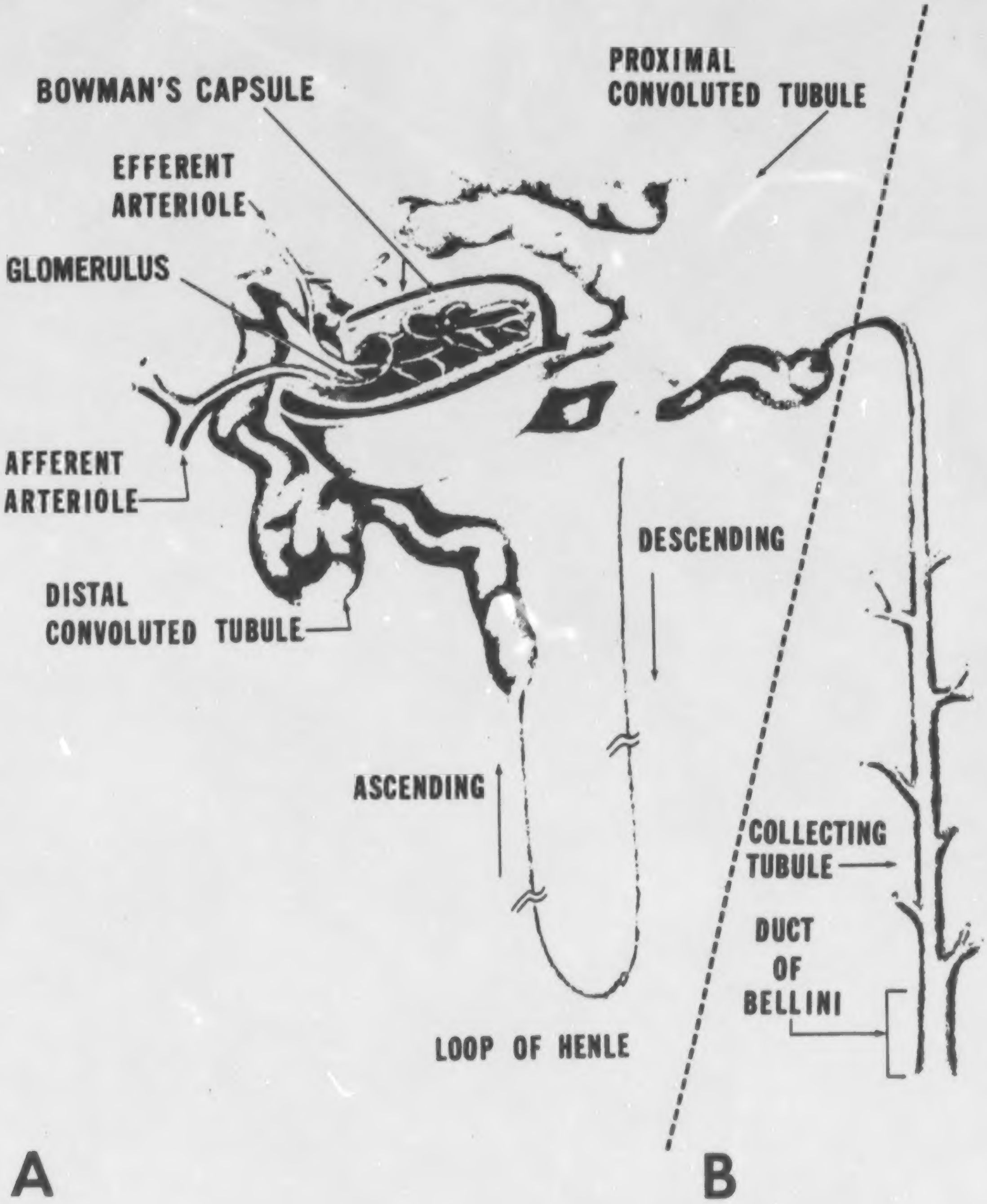
BOWMAN'S CAPSULE
PROXIMAL CONVOLUTED TUBULE
EFFERENT ARTERIOLE
GLOMERULUS
AFFERENT ARTERIOLE
DISTAL CONVOLUTED TUBULE
DESCENDING
ASCENDING
COLLECTING TUBULE
DUCT OF BELLINI
LOOP OF HENLE
A
B

Figure 6. The nephron unit and collecting tubule.

150 to 250 microns in diameter, and there are about 1,500,000 glomeruli in each kidney as previously mentioned. Each glomerulus consists of Bowman's capsule and Malpighian tuft. Bowman's capsule is a double-walled epithelial sac which surrounds the tuft and receives glomerular filtrate. The proximal convoluted tubules are about 14 mm. in length and are lined with structurally similar cells. (These cells do not all have the same function, however.) The cells of a proximal tubule are quite different from those lining the descending limb of the loop of Henle. The ascending limb of the loop, which leads into a distal convoluted tubule, is somewhat larger than the descending limb. The distal tubule is approximately 5 mm. in length and 20 to 50μ in diameter. The cells lining the distal tubules are quite characteristic of that portion of the tubules. Although not part of the nephron unit, a system of collecting tubules begins in the cortex and eventually unites with other tubules in large collecting tubules labeled *ducts of Bellini* in figure 6,B. Cells lining the collecting tubules are unlike those lining secretory tubules. Histopathologists are very much aware of pathological changes which occur in cells of the tubules under various conditions. Tubular cells have recently been studied in detail with the electron microscope. It has been demonstrated that many biochemical processes take place in cells of the tubules, partly by relating the presence of structures shown with the electron microscope to their known activities in other cells. This is an example of how knowledge of structure can lead to an understanding of function. Obviously morphology alone does not supply all of the information desired. With this in mind, we will now turn our attention from structure to function.

4. Physiology of the Kidney

4-1. Most laboratory technicians in the Air Force analyze many urine specimens every day. Yet, would most of us be able to explain how the body produces urine? Even more important in laboratory work, can we relate how physical properties of urine, e.g., color, specific gravity, etc., have any real practical relationship to kidney function? If we are to describe urine and list its properties, it is only reasonable that we know why we are doing all of these tests. This section describes functions of the kidney and relates them to urine production.

4-2. As a result of normal cellular metabolism, a number of nitrogenous wastes are formed. These include urea, uric acid, creatinine, and ammonia. Removing these wastes from the blood is one function of the kidney. It also regulates blood volume, controls the water content of tissue, and maintains the pH and chemical composition of body fluids. The process of urine formation begins with filtration of plasma-like fluid through the glomerular capillaries. The filtrate then passes down the tubules. It is reduced in volume and composition by tubular reabsorption and secretion which we will discuss in somewhat greater detail.

4-3. An important function of the kidney is glomerular filtration. Blood pressure of approximately 70 mm. of mercury within the capillaries of Bowman's capsule is about twice that of any other capillary in the body. Opposing this force is an osmotic pressure of nearly 25 mm. of mercury, which is normal capillary pressure. About 10 percent of the blood which passes through the glomerulus is removed in the form of glomerular filtrate. Approximately 1200 ml. of whole blood pass through the kidneys each minute, thus, every 4 or 5 minutes a volume exceeding that of the total volume of blood in the body passes through the kidneys. Capillaries of the glomerulus are concerned only with filtration, not with reabsorption as are other capillaries of the body. Various mechanisms affect the rate of glomerular filtration. In the absence of compensating factors, a rise in blood pressure in the efferent arteriole increases the rate of filtration. This may be due to vasoconstriction of the efferent arteriole and possibly to an increase in systemic blood pressure. Glomerular blood flow is also under neutral control. For example, stimulation of the splanchnic (visceral) nerve decreases renal blood flow and, hence, reduces the rate of filtration. Vasodilators such as adrenalin also decrease glomerular filtration after an initial phase of vasoconstriction.

4-4. In the actual process of filtration, walls of the glomerular capillaries behave as filters. A very thin membrane, 0.1 μ thick, separates the blood from the cavity of the capsule. Substances having a molecular weight of 68,000 or less are able to pass through the membrane and are excreted. Proteins are normally retained. Thus, you can see why albuminuria (albumin in the urine) is generally considered abnormal. If small amounts of protein do pass through the glomerulus, the protein is reabsorbed in the proximal convoluted tubules. The pH of glomerular filtrate is about 7.4, with a normal specific gravity of 1.008 to 1.012 (aver. 1.010).

4-5. Most reabsorption takes place in the renal tubules. Many substances, including water, which are essential to life escape through the glomerular tuft. These substances must be replaced in the blood. In order to return them to the bloodstream, the convoluted tubules actively reabsorb some of these substances into the capillary network around the tubules. The proximal tubules reabsorb all of the glucose and 85 percent of the water from glomerular filtrate and about the same amount of sodium, bicarbonate, and chloride. If the plasma concentration of a solute exceeds a certain level known as the tubular maximum, this substance appears in the urine. Other substances which are absorbed include amino acids and phosphates. Additional water reabsorption is thought to occur in the loop of Henle. After this, approximately 20 to 40 percent of the water remaining in the glomerular filtrate is reabsorbed in the distal tubules. Reabsorption in the distal tubules is under hormonal control, namely the antidiuretic hormone (ADH) of the pituitary gland.

4-6. In addition to reabsorption, the tubules excrete many substances including creatinine, ammonia, potassium, and foreign substances like penicillin and dyes. Most potassium found in the urine is excreted by the tubules, with the remainder having escaped in the glomerular filtrate without reabsorption. Incidentally, potassium is thought to be the only electrolyte which is actively excreted by the tubules. As the glomerular filtrate goes through these processes of filtration, reabsorption, and secretion, the total volume drops from the original 100 to 150 liters or more per 24 hours to about 1 to 1.5 liters of urine per 24 hours. The pH of urine is 4.6 to 8, while the average specific gravity increases from the original 1.010 to about 1.015 upward to 1.025.

4-7. Our discussion of kidney physiology would not be adequate without mentioning the importance of the kidneys in maintaining acid-base balance.[2] Regulation of acid-base balance is accomplished by four mechanisms, not necessarily in order of importance. First, ketone bodies (acetoacetic acid, B-hydroxybutyric acid and acetone) which may be present are oxidized to corresponding organic acids. These acids are subsequently excreted after conjugation with glycine as hippuric acid or phenylaceturic acid. By this means ketone bodies which cause acidosis are removed from the blood and excreted. Second, ammonium ions replace the more basic sodium ions to some extent. Ammonia from plasma amino acids and glutamine are released by enzymatic activity in the tubular cells of the kidney. Thus, losses are conserved by retention of sodium and substitute excretion of ammonium ions. Third, reabsorption of bicarbonate takes place in the tubules as previously indicated. Fourth, hydrogen ions are excreted by the tubules. The overall result is a change in the pH of glomerular filtrate from 7.4 to a range of 4.6 to 8.0 while the acid-base balance of the body is maintained. The latitude of urine pH from acid to alkaline is to be expected and results from compensating mechanisms which control blood pH within a very narrow range.

5. The Kidney and Disease

5-1. Our attention will now be focused on some of the pathological conditions which either cause or are related to malfunction of the kidneys. As a student, your objective in studying this section is to realize what an abnormal laboratory report can mean to the physician and, thereby, encourage you to render valid and intelligent reports. As repeated often in this course, clinical conclusions relative to a particular patient are beyond the scope of the laboratory.

5-2. You have on occasion seen the term *uremia* in the clinical block of laboratory report forms and elsewhere. It is a rather nonspecific term indicating the presence of abnormal quantities of urinary constituents in the blood. Uremia may result from practically any disease involving a malfunction of the kidneys and is represented by complex clinical symptoms. When uremia is diagnosed, provisionally or definitively, the laboratory is used to reinforce the diagnosis. In most cases there is an elevation of urea nitrogen in the blood and oliguria. However, not all of these signs and symptoms are always present. For example, the quantity of urine may be normal, in which case the serum sodium is usually increased. In the final analysis, laboratory data are meaningless unless one laboratory result is related to another. There is sometimes a question concerning the relative merits of particular procedures. If a choice exists between measuring urea nitrogen and nonprotein nitrogen, it would be advisable to select the urea nitrogen test because of its greater specificity. Nonprotein nitrogen measures creatinine, amino acids, and a composite of nitrogen containing substances, as you may recall from your study of clinical chemistry.

5-5. There are many diseases of the glomeruli. There are also many recognized classifications of glomerular diseases. The type of glomerular disease is diagnosed partly on histological and partly on other laboratory evidence. For example, some glomerular diseases are accompanied by hematuria (blood in the urine) or proteinuria while others are not. This again emphasizes the need for careful and accurate laboratory work. You might also recognize that medical terms like glomerulonephritis have a limited and specific meaning. The term is not used to describe just any disease of the glomerulus. This is mentioned as a word of caution to urge you to form a meaningful medical vocabulary. You undoubtedly recognize that diseases not generally considered renal in nature may result in renal complications. For instance, nearly half of all patients with systemic lupus erythematosis (L.E.) have renal complications.

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[2] Acid-base balance. This is a term widely used throughout literature and is explained in Chapter 1, Volume 2, of this course. It refers to an equilibrium state between acidic and basic ions in order to maintain the pH of blood very near 7.4; disturbances in acid-base balance may result in acidosis or alkalosis.

5-4. A variety of kidney disorders are classified as diseases of the tubules. Included in this category are mercury poisoning, gout, and other conditions, especially effects relating to toxicity. Also included among tubular disorders is multiple myeloma. Multiple myeloma is a malignant, tumorous condition of the bone marrow manifested in 40 to 50 percent of the cases by Bence Jones protein in the urine. However, myeloma is more than a disease of the bone marrow. Involvement extends to the spinal cord with consequent pyelonephritis. In addition, renal lesions develop although there is less certainty on the causal relationships between renal lesions and myeloma. Renal amyloidosis (starch-like deposits) is another aspect of this disease. The molecular weight of Bence Jones protein is in the range of 25,000 to 90,000. It, therefore, passes readily through the glomerulus if present and can be easily detected in the laboratory.

5-5. There are a variety of basic renal problems not previously discussed. In recent years there has been a resurgent interest in the effect of infections upon functions of the kidney. Infection may produce generalized and localized kidney damage, including interstitial nephritis and pyelonephritis. Typhoid, yellow fever, and tuberculosis are just a few diseases of special interest to a urologist. Another quite different problem which was developed in your study of clinical chemistry is the occurrence of renal calculi. Renal calculi are not as uncommon as you might think. They have been reported to occur in 5.4 percent of all autopsies, which is a good indication of their occurrence in the population. Next let us mention tumors and malignant neoplasms such as lymphatic leukemia to illustrate that other conditions have a profound influence on kidney pathology. Unless you are a histopathology technician, you will probably not directly observe the effects of these conditions on tissue. However, the effects will be apparent from other tests which you will perform.

5-6. Finally, in this category of miscellaneous diseases which affect the kidneys can be included diseases of the blood vessels and diabetes, a disease which you studied in Chapter 4, Volume 2, of this course. There are at least a dozen well-known vascular diseases which include glomerulosclerosis, nephrosclerosis, arteriosclerosis, and thrombosis. These usually involve the capillaries, arterioles, arteries and veins, respectively. In diabetes mellitus a high blood sugar results from insufficient production of insulin by the pancreas. When the glucose level of the blood exceeds that which can be reabsorbed by the tubules, the renal threshold has been exceeded and glucose appears in the urine. Kidney damage results from accompanying effects of the disease. Keep in mind that glucose in the urine is only one of various symptoms.

Physical Characteristics of Urine

A REPORT CONCERNING the physical characteristics of urine is a part of every complete urinalysis. The macroscopic examination includes total volume, color, appearance, pH, and specific gravity. In this chapter we will discuss all of these tests and their physiological significance. We shall also describe techniques generally used in the laboratory to evaluate the physical characteristics of urine.

6. Urine Volume, Color, and Appearance

6-1. **Urine Volume.** The total urine volume voided in 24 hours varies with diet, body size, fluid intake, fluid loss in perspiration from temperature or exercise, and the ingestion of diuretics, such as coffee. The average total daily volume is about 1,200 to 1,500 ml. from 10 years of age through adulthood. However, in children the total volume in relation to body size is increased considerably. The maximum total daily output of urine should not normally exceed 2,000 ml. in adults. Volumes higher than this, *polyuria*, are associated with pathologic conditions such as diabetes insipidus, diabetes mellitus, certain kidney diseases, and neuroses. A decreased total urine output, *oliguria*, results from lowered fluid intake or loss of fluid from diarrhea, vomiting, fever, and hemorrhage. There is normally a decreased total urinary output during the night, probably due to decreased intake of fluid.

6-2. Random specimens are most often requested for routine urinalysis. The volume varies considerably, but this is unimportant as long as the minimum amount (usually 15 ml.) is obtained. A fresh, concentrated, first-morning specimen is undoubtedly the most valuable specimen to the physician. This is particularly true in regard to the formed elements, i.e., cellular components. The specimen should never be discarded as QNS (quantity not sufficient) without performing as many of the requested tests as possible. This usually includes all of the routine examination, except perhaps the specific gravity. However, even the specific gravity can be determined as long as the volume is sufficient to dilute. The quantity of urine obtained for analysis is a particular problem with children and infants. As a technician you have a responsibility to assist parents in obtaining an adequate specimen. However, other laboratory tests (i.e., blood count, etc.), should not be delayed because you must wait to obtain a urine specimen.

6-3. **Color.** Urine color varies considerably in healthy and diseased states. Normally, urine is some shade of yellow. This yellow may be almost colorless, yellow-green or straw, pale yellow, bright yellow, light amber, or dark amber. Variations in body metabolism and pigments from the diet will alter these colors. Chromogens in beets, for instance, may produce red urine; while carrots give a bright yellow color (carotene) to the specimen. These pigments may be so concentrated that they confuse color comparisons made in urine strip screening tests, e.g., Labstix®, Tes-tape®. In this case several alternatives are possible. Chromogens may be removed by mixing with activated charcoal and filtering. This filtrate can then be screened for glucose. However, since normal chromogens are also removed by activated charcoal, the pH is probably altered in this process and protein analysis may be invalidated. Qualitative protein analysis could be determined with heat and acetic acid on the untreated specimen. It may also be stated that light-colored specimens are usually lower in specific gravity than darker specimens. There are exceptions, though, as in the case of diabetes, where the urine may be straw or pale yellow in color and yet have a high specific gravity due to the presence of glucose.

6-4. Pathological urine specimens, then, may have the same color as normal urine. A green-yellow specimen may result either from normal metabolism or from the presence of bile or bacterial chromogens, i.e., *Pseudomonas* infection. Red urine is produced from medications like PSP (phenolsulfonphthalein) dye or pyridium, which are often used in kidney disease diagnosis or therapy. Red color may also be caused by the presence of intact red blood cells (hematuria) or hemoglobin drived from red cells (hemoglobinuria). Porphyrins impart a wine red color to urine (porphyuria). Remember, though, a red color can appear after the ingestion of beets. Alkaptonuria is characterized by the presence of alkapton bodies (homogentisic acid) and a consequent black urine when the urine specimen is made alkaline or becomes alkaline upon standing. Alkaptonuria occurs because of an inborn error in the metabolism of two amino acids, phenylalanine and tyrosine. Black urine is seen also when melanin, the pigment of hair and skin, is present as the result of certain malignancies. Finally, pus, certain crystals, chyle (fat), and some bacteria give a milky appearance to urine.

6-5. Definite assumptions as to the presence of pathology cannot be made solely on the basis of the color of urine. However, abnormal urine color should alert you to the possibility of pathology which may be confirmed or denied by more specific tests, and the physician's findings in his examination of the patient.

6-6. **Appearance.** The appearance of urine refers to another physical characteristic which is routinely observed and reported. The term "apperance" refers to the transparency of the specimen. Clear, hazy, and cloudy are descriptive terms used for this report. Suspended crystalline particles of phosphates, urates, and carbonates are often the cause of haze or cloudiness. However, heat and acetic acid may be used to dissolve urates and phosphates respectively. Cellular material including epithelial cells, blood, pus, and bacteria will also cloud the urine speciemen. These substances, as well as insoluble crystalline salts, can be removed by centrifugation. Confirmation of the nature of the material causing cloudiness is usually done microscopically. Rarely, chyluria (fat in the urine) may cause a specimen to appear cloudy, and this is also confirmed microscopically. Chyle is easily removed by (1) adding ether to dissolve the fat and (2) separating the aqueous/ether phases in a separatory funnel.

7. Reaction and Specific Gravity

7-1. **Reaction.** The reaction (pH) of freshly voided normal urine varies from 4.8 to 8.0 with a mean of approximately 6.0. As the specimen stands at room temperature urine becomes less acid (pH becomes numerically higher) by the formation of ammonia from urea, which is a normal chemical constituent of urine. The urea is chemically split by bacteria to form ammonia. The concentration of ammonia in old urine specimens can become so high as to be noticeable from its distinctive odor. A marked pH shift to alkaline in aged urine interferes with certain qualitative screening tests, i.e., protein stick tests, and destroys important microscopic cellular components. This is the principal reason for performing a routine urinalysis only on a fresh urine specimen. The pH is of clinical diagnostic significance only in a freshly voided or catheterized specimen.

7-2. Strongly acid urine (i.e., pH lower than 6.5) is encountered in cases of metabolic acidosis—the ketosis of diabetes mellitus, for example. In addition, strongly acid urine reactions are found in gout, acute rheumatism, chronic nephritis, tuberculosis of the kidney, fever, leukemia and inflammations of the heart, liver, kidneys, and lungs. Dehydration causes an acid reaction because of the loss in liquid volume without a simultaneous reduction in acid (hydrogen ion) excretion by the kidneys. Finally, an acid reaction in urine may occur from the ingestion of a high-protein diet.

7-3. Urinary tract infection is the most common cause of persistent strongly alkaline urine. Again, this occurs because of the ability of urinary bacterial pathogens to split urea to form ammonia. The microorganism *Proteus vulgaris* is most frequently identified in this connection. An alkaline reaction is also found with persistent vomiting (gastric acidity is reduced) in certain anemias, some cases of debility, cystitis, and in cases of obstructive ulcers.

7-4. As you noted in the preceding paragraphs, urinary pH may be altered by several pathological processes. The physician may also intentionally alter the urine pH in conjunction with therapy. This is generally the situation in patients suffering from the formation of urinary calculi. You learned in Volume 3 of this course about the occurrence of urinary calculi, and that certain calculi, specifically oxalate, uric acid, and cystine stones, form in an acid urine. In treatment, then, the physician adjusts the urine pH through proper diet and drugs to maintain a persistently alkaline urine. This eliminates the chemical environment in which these acid stones form. Conversely, phosphate and carbonate stones, formed in alkaline urine, are treated by creating in the urinary tract an acid urine medium which inhibits their formation. Another instance in which the physician controls urine pH is in the treatment of a urinary tract infection with the antibiotic, streptomycin. Streptomycin is effectively bacteriostatic only when an alkaline pH is maintained. In therapy with the sulfonamides an alkaline urine is created to prevent precipitation of sulfa crystals and consequent damage to the urinary tract.

7-5. As you have seen, urinary pH can be a helpful indicator for some physiological functions, and it adds one more parameter to the physician's clinical picture of a patient. Also, we noted that in some instances urinary pH measurements are essential for adequate treatment of the patient. Of course, your responsibility to the clinician and his patient is to know the technical aspects of urinary pH measurement and to be able to determine pH accurately and quickly.

7-6. **Urine pH Measurement.** For many years urine reaction was measured only with litmus paper. The color change from red (acid) to blue (alkaline) in litmus occurs at a pH of approximately 6.5 units. Therefore, the reagent permits only a qualitative reading, i.e., the urine specimen is either acid (below pH 6.5) or alkaline (pH above 6.5). It is clear from our previous discussion of the correlation between physiological state and urinary pH that such a crude measurement is of little value in clinical evaluation or treatment. Universal indicators or mixed indicators are much more useful because they indicate pH throughout the range of urine pH values. Several types of pH strip indicators are shown in the training aids package. You can see that there are distinguishing colors at specified pH levels.

7-7. Paper strips impregnated with a variety of indicators are available. The pHydrion® paper strips are furnished through Federal supply channels (FSN 6630–442–9005). As you have seen on the pHydrion indicator, the pH range of these indicator strips is from pH 1 (red) through pH 11 (blue) with color changes for each whole numbered unit. The strips may be used for any rough pH approximation. They are not made exclusively for use in urinalysis. This is the reason that such a wide range of pH values is available on a single strip. Several sources of error must be kept in mind when you use these strips. First, the strips should be kept in the sealed dispenser and away from strong acid or alkaline fumes when not in use. Secondly, the strips should be kept dry until they are used. Then, when you actually measure pH, you should not soak the indicator strip in the solution you are testing. Obviously, this means that you should not drop an indicator strip into the urine. If the strip is soaked in urine the indicator reagent will be leached from the paper and pH will be impossible to read. The correct way is to dip one end of the strip into the urine. Dip it only long enough to saturate the end portion, and drain it immediately by touching the strip to the side of the container. The strip should not be placed on the lab bench after wetting because of a good possibility of contaminating the reaction with residual chemicals on the bench. Sanitation of the workbench is another consideration.

7-8. The Labstix® and indicator, also included in the training aids package, is designed exclusively for qualitative and semiquantitative measurement of specific constituents in urine. The innermost band on the Labstix contains the pH indicators, methyl red and bromthymol blue. The original color is orange (pH 5), and the range is through pH 9, which is a blue color. Each Labstix is a strip of cellulose with a series of small squares of reagent-impregnated paper attached. The cellulose is nonwettable and therefore easily drained, so that the reagent squares are not soaked by an excess of specimen. In addition to the sources of error mentioned in the previous paragraph it should be noted that these strips are packaged in a screw-capped bottle which contains a desiccant (drying) chemical. The bottle must be kept tightly closed if the reagents are to remain dry and stable. Also, the desiccant packet must be left in the storage bottle. If these precautions are not observed, atmospheric moisture will be absorbed by the strips and the reagents will deteriorate.

7-9. **Specific Gravity.** "The ratio of the weight or mass of a given volume of a substance to the weight of an equal volume of a standard, i.e., pure water," defines specific gravity. For practical purposes, 1 cc. (cm.^{3}, cubic centimeter) of pure water weighs 1 gram (1.000 g.) at 4° C. To illustrate this definition, let us suppose we have an exact cc. of a substance such as urine and weigh it accurately at 4° C. to three decimal places. If this cc. of urine weighs 1.010 g., then we can assume by the definition that the sp. gr. is a ratio of $\frac{1.010}{1.000}$ and the sp. gr. is 1.010. Actually this procedure is impractical for routine use with multiple specimens. However, another way to determine specific gravity is to measure the displacement of a fluid by a solid of constant weight immersed in the fluid. This is the principle of the urinometer (hydrometer) pictured in figure 7.

7-10. The urinometer is calibrated in distilled water at the temperature specified on the stem by the manufacturer. The sp. gr. of distilled water at the calibration temperature should be 1.000. For each 3° C. of specimen temperature above the calibration temperature 0.001 is added to the sp. gr. reading obtained. Of course, this value is subtracted for each 3° C. less than calibration temperature. All new urinometers should be checked for accuracy and mathematical corrections should be made for slight inaccuracies.

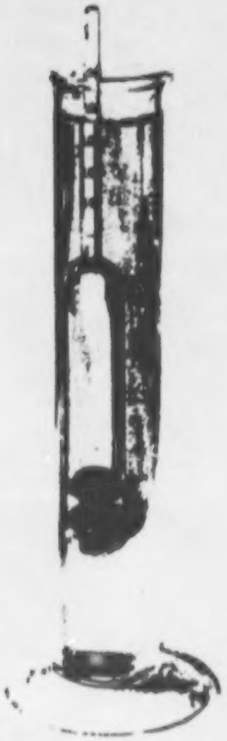

Figure 7. Urinometer (hydrometer).

If the calibration value is grossly inaccurate, the urinometer should be discarded.

7-11. The technique of using a urinometer is quite simple. First, bring the urine specimens and water for calibration to room temperature. If this is not done, the temperature of each specimen should be determined at the time the sp. gr. is read and suitable correction made. After this, a volume of specimen sufficient to float the urinometer is placed in the glass cylinder. The urinometer must not touch the bottom of the cylinder. Then spin the urinometer in the urine and take a reading at the bottom of the meniscus before the urinometer stops turning and settles to the side of the cylinder. Read the result to the nearest 0.001 unit. Then add or subtract the calibration value and record the sp. gr.

7-12. The minimum volume of urine necessary to float a urinometer is approximately 15 ml. This varies somewhat, depending upon the sp. gr. You should calculate the dilution to be made by measuring the volume of urine available. For instance, if 10 ml. of specimen are obtained, a twofold dilution with distilled water results in a final volume of 20 ml. (10 ml. urine + 10 ml. H_2O). This is more than 15 ml. and sufficient to float the urinometer. Fo a twofold dilution the three digits following the decimal point must be doubled when the sp. gr. is read. For instance, if the reading on the urinometer is 1.004 after correction for temperature, the actual sp. gr. would be 1.004 + 0.004 or 1.008. If the specimen is diluted threefold (i.e., 5 ml. urine + 10 ml. distilled water), then the last three digits would be tripled. Thus, a temperature corrected reading of 1.003 on a threefold dilution would mean a sp. gr. of 1.009 for the undiluted urine. The glass cylinder may be rinsed with tap water between specimens and *must* be rinsed after very cloudy specimens to facilitate later readings.

7-13. Problem Situation #1: Assume that you have a urinometer which was originally calibrated at 15° C. You check this calibration at 20° C. (room temperature) in distilled water and obtain a reading of 1.002 on the urinometer. Your first urine specimen is at room temperature and it reads 1.010. What is the sp. gr. of this specimen?

7-14. Problem Situation #2: Your second specimen is freshly voided urine for a concentration test and you read it immediately while it is warm (37° C.). The reading on the urinometer is 1.020. What is the sp. gr. of the specimen?

7-15. Solution to Problem Situation #1: Your urinometer was calibrated to read 1.000 at 15° C. You checked your calibration at 20° C., which is 5° C. higher than calibration temperature. For each 3° C. higher you must add 0.001 to the observed reading.

$$5^\circ \text{ C.} \div 3^\circ \text{ C.} = 1.7$$
$$1.7 \times 0.001 = 0.0017$$

This is rounded off to 0.002, which is added to 1.000 and gives the calibration result you read at 20° C. or 1.002. You must add this value, 0.002, to all readings made at this temperature. Your first urine specimen read 1.010 at 20° C., therefore, you add 0.002 and the sp. gr. is actually 1.012.

7-16. Solution to Problem Situation #2: In this test for urine concentration the normal specific gravity should exceed 1.022. A sp. gr. result of 1.020 might be considered equivocal or below normal. However, you must consider that the urinometer calibration read 1.000 at 15° C. and you read the warm urine at 37° C. This is a 22° C. difference in temperature. In paragraph

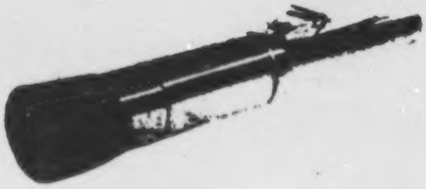

Figure 8. Clinical model refractometer.

7-10 you learned that 0.001 must be added to the observed value for each 3° increment higher than the calibration temperature.

$$22^\circ \text{ C.} \div 3^\circ \text{ C.} = 7.3$$
$$7.3 \times 0.001 = 0.007$$

Adding 0.007 to the urinometer reading will make the sp. gr. 1.027. This result is much less equivocal than the original reading and it is accurate.

7-17. **Refractometric Measurement of Specific Gravity.** There is a direct relationship between the concentration of dissolved solids and the refractive index of a solution. By definition, specific gravity is a ratio of the weight (or mass) per unit volume, so specific gravity is also related to the refractive index of a solution. A refractometer is an instrument designed to measure refractive index. A small clinical refractometer developed in recent years has replaced the urinometer in many laboratories. Figure 8 is a photograph of the clinical model refractometer. This instrument will accurately measure urine specific gravity to the nearest 0.001 unit. It has an advantage in that it requires only 0.02 ml. of specimen and is easily loaded with urine. Because of the small sample size, the specimen assumes ambient (room) temperature immediately and the corrected value can be read directly from an internal scale. Calibration is easily checked against the refractive index of water. The efficiency in time saved in taking multiple specific gravity readings is a great asset with this instrument. Of course, freedom from tedious manipulations is also advantageous. Special care should be exercised in using this delicate optical instrument. Holders are available to help prevent accidentally dropping the refractometer while it is in use. If a holder is not used, the instrument should be stored in the case provided by the manufacturer. In no circumstance should the refractometer be left on the lab workbench where it might be damaged or pilfered.

CHAPTER 4

Microscopic Examination

IN CHAPTER 1 we learned the proper method for establishing critical microscopic illumination. We will now discuss some of the important components of urine which can be observed and identified with an adequate microscope system. The biological origin of these components and their relevance to health and disease relate to the structure and functions of the human excretory system described in Chapter 2. Our attention now turns from the gross physical features outlined in Chapter 3 to a systematic examination of urinary sediment as another valuable tool to aid the physician in diagnosing and treating the patient.

2. The urine specimen must be fresh since the nature of the sediment changes with time. The pH changes as mentioned in Chapter 3 and as a result, cellular elements disintegrate. Also, dissolved materials which precipitate not only change the specific gravity but obscure the microscopic field. As urine stands, unstable chemical components such as urobilinogen will deteriorate until they can no longer be detected. It is essential that the microscopic examination be performed within a few hours after collection in order to obtain valid results. Refrigeration and/or adding formalin is required if a delay in the analysis cannot be avoided.

3. In addition to these important points which were discussed in the preceding chapters, it is recognized that technicians may not possess adequate knowledge of structures present in the sediment. In this chapter we will consider both cellular (organized) crystalline and amorphous urinary sediments. The use of stains in obtaining consistent and reliable microscopic results will be emphasized. Also included is the general significance of normal and abnormal findings.

8. Organized Sediment

8-1. Body cells and their derivatives are referred to as *organized sediment*. The common types of organized sediment include blood cells, epithelial cells, spermatozoa, and casts. Such structures may be found in small numbers in most urine specimens but if present in large quantities, they usually indicate a pathological condition. Other organic materials which may be found in urine include mucus, bacteria, yeasts, parasites, and fat globules.

8-2. Various contaminants often resemble organized sediment. Talcum powder, starch granules, and oil droplets are sometimes mistaken for blood cells. Starch granules vary in size and shape and turn blue-black upon addition of iodine. Oil droplets are spherical and show concentric rings of light refraction upon focusing up and down with the fine adjustment. Pollen grains may be confused with erythrocytes or parasites. They vary in size and appearance according to their source. Generally such plant material can be distinguished from animal cells by the comparatively thick cell wall and lack of organized internal morphology. Yeast from urine bottles can confuse a microscopic examination, although the use of disposable paper containers reduces the potential problem. Yeast cells can usually be identified by their characteristic budding. Even air bubbles may be mistaken for cells. It is helpful to rotate the eyepiece periodically to be certain that extraneous structures adhering to the glass are not being identified as objects in the specimen.

8-3. **Preparing the Specimen.** In most hospital laboratories, large numbers of specimens for microscopic examination arrive in the laboratory in groups and individually throughout the day. There is generally no reason to hold these specimens or preserve them. Perhaps the greatest problem is keeping them properly identified and separated from "stat" requests. In a well-organized laboratory, urinalysis will begin with the first group of specimens which can be conveniently processed. In other words, don't wait until 0900 hours to examine specimens which arrive at 0700 hours. Formed elements may disintegrate and valuable information will be lost.

8-4. Is it necessary to centrifuge all specimens for microscopic analysis? While some workers contend that it is *not* desirable to centrifuge urine specimens, experience has shown that centrifugation is probably the best approach. There is a chance that not all significant sediment will be observed on direct examination of urine. Centrifugation will not destroy fragile cells if the specimen is not centrifuged excessively. On the other hand, relatively rare elements, such as casts, may be missed altogether if the specimen is not centrifuged. In most laboratories, it is considered best to centrifuge 10 ml. of urine and report all elements except casts in number per high power field. Casts are reported in number per low power field. It is left for local policies to decide which specimens, if any, can be observed directly without centrifugation. Cover slips should be used to enhance uniform distribution of the sediment which is transferred from the centrifuge tube to a corresponding slide. The entire specimen must be thoroughly mixed before you pour a portion of it into a centrifuge tube. There should never be more than two specimens on any one slide. The use of Boerner slides is not recommended because these slides do not permit the proper use of cover slips.

8-5. Urine may be cleared of amorphous carbonates and phosphates by adding a few drops of dilute acetic acid (less than 10 percent) to 10 ml. of urine before centrifuging. This will permit a clear view of significant structures. However, be careful not to add more acid than necessary to clear urine because casts and erythrocytes may also dissolve. The urine may be cleared of amorphous urates by mixing the urine with equal parts of warm (38° C.) physiological saline before centrifuging. If less than 10 ml. of the urine are centrifuged, you will need to make a correction for dilution. Of course, if you use 10 ml. of urine in addition to diluent, no correction is necessary.

8-6. **Staining Urinary Sediment.** Before discussing the appearance of structures in the urine, we must decide whether we will be viewing stained or unstained sediment. The use of stain is recommended because it permits rapid and accurate identification of organized sediment. There are no real objections to using a stain except lack of training in this regard on the part of the technician. Best known of the supravital stains is the so-called Sternheimer-Malbin stain. The working stain consists of three parts solution A and 97 parts solution B, and is stable for several weeks if it is filtered as required. Stock solutions A and B are stable indefinitely when kept in separate containers, and consist of the following ingredients.

Solution A

1. Methylrosaniline chloride (crystal violet)—3.0 g.
2. Ethyl alcohol 95 percent—20.0 ml.
3. Ammonium oxalate 0.8 g. and distilled pure water to 80 ml.

Solution B

1. Safranine 0—0.25 g.
2. Ethyl alcohol 95 percent—10.0 ml.
3. Distilled water to 100 ml.

The stain is not difficult to use since it can be kept in a dropper bottle and a few drops can be added to the sediment in the centrifuge tube. Once you are familiar with observing stained sediment, it will be easy to observe and identify the various types of organized sediment. As emphasized in Chapter 1 of this volume, without critical illumination it is virtually impossible to accurately perform a urinary microscopic examination. Some of the difficulties associated with illumination can be overcome by staining the sediment. Throughout the remainder of this chapter, we will describe sediment in both the stained and unstained conditions.

8-7. **Epithelial Cells and White Blood Cells.** We will not attempt to distinguish among the types of epithelial cells on the basis of pathology alone. It is now believed that it is difficult if not impossible to state where in the urinary tract epithelial cells originate.[3] You should be extremely cautious in attempting to define the origin of a particular cell. The term "renal epithelial cell" is often used to indicate a rounded type of cell. The term "renal" may be a misnomer in some cases because these cells do not always come from the kidney. Various types of epithelial cells are shown in detail A of foldout 1, which you will find at the back of the volume. However, it should not be inferred that cells which appear rounded necessarily had their origin in the kidneys. On the other hand, it is true that large numbers of these cells will be found in chronic kidney disorders, in nephritis and in nephrosis. When epithelial cells are noted in casts, they are presumed to arise from the kidney tubules.

8-8. It is difficult to state the exact significance of epithelial cells in the urine. A few epithelial cells will be present in every specimen. Generally, there will be more epithelial cells in specimens from females than in specimens from males. Partly because of the predominance of vaginal epithelial cells in many specimens, it is advisable to obtain "clean catch" specimens. These epithelial cells from the vagina are of the squamous variety and have no significance in the study of renal disease. Squamous cells are large and flat with round nuclei and may be cornified or uncornified. Ordinarily, no effort need be made to describe or classify them. If it becomes necessary to further describe epithelial cells, the Sternheimer-Malbin stain is very helpful.

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[3] Lippman, Richard W., *Urine and the Urinary Sediment*, Chas. C. Thomas Publisher, Springfield, Ill. 1964.
Davidsohn, Israel, and Wells, Benjamin B., *Clinical Diagnosis by Laboratory Methods*, W. B. Saunders Co., London 1962, 13th ed.

8-9. Far more important than epithelial cells, from a clinical standpoint, are the white blood cells. A few leukocytes are present in normal urine, although it is for the physician to determine how many is normal. Increased numbers of leukocytes may originate from any part of the genital or urinary tract. When these cells are present in great numbers, especially neutrophiles, they are sometimes referred to as *pus cells*. There is no particular advantage in using this term, and instead the report should be given as the number of WBC's per hpf (high power field).

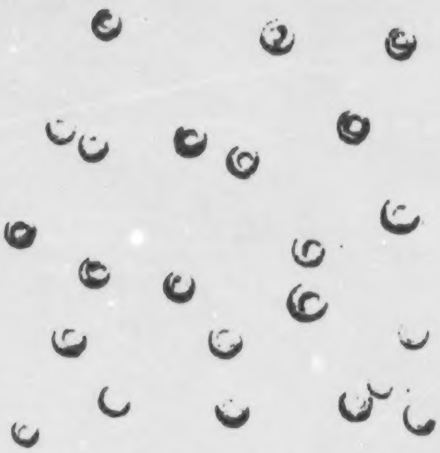
8-10. White blood cells are quite easily distinguished from red blood cells on the basis of size and the presence of internal structures. Obviously, white blood cells are larger, and unless accompanied by at least 5 or 6 red cells per hpf, will not give a positive benzidine test. Occasionally a white precipitate due to the presence of albumin will obscure the nuclear structure of the leukocytes. The addition of dilute acetic acid will clear the field and enable you to see the nuclei. It is important that red blood cells be identified before adding the dilute acid. Unless you do observe the nuclei, it is very difficult to distinguish white blood cells from rounded epithelial cells.

8-11. The presence of pus in urine is termed *pyuria*. In alkaline urine the leukocytes will adhere to each other in clumps. For an accurate count of those cells, the urine is acidified and a blood counting chamber is used. Qualitative or quantitative tests for albumin should be performed on the clear centrifugate which will be stressed again in the following chapter. It is often of diagnostic importance to know if proteinuria (protein in the urine) is occurring in addition to that derived from the pus cells themselves. For example, when pus cells are found without proteinuria, it is unlikely that the infection involves the renal parenchyma (functional tissue). The major causes of pyuria are tubular and pyogenic infections, tumors, and trauma.

8-12. Before concluding our discussion of epithelial cells and leukocytes, we will recognize two particular kinds of cells, the *oval fat body* and the *glitter cell*. Oval fat bodies are characteristic of degenerative tubular disease. They are found within the intact epithelial cell periphery as inclusions (fat droplets). Oval fat bodies may appear black under low illumination because of their high refractive index. They stain orange with Sudan III. Glitter cells are nonviable, polymorphonuclear leukocytes which contain many bacteria. They may be confused with epithelial cells that contain fat. Their name is derived from the glittering, shining appearance in moderately bright light. The diagnostic value of glitter cells is uncertain. They are reported to be of some diagnostic value in cases of pyelonephritis. With the Sternheimer-Malbin stain, glitter cells stain pale blue, whereas other white blood cells stain darker blue. White blood cells, glitter cells, and oval fat bodies are pictured in detail B of foldout 1.

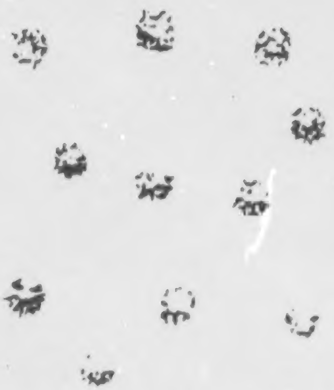
8-13. **Hematuria and Erythrocytes.** The appearance of blood in the urine, whether gross or only a few cells, is referred to as *hematuria*. Hematuria may be due to any one of several clinical conditions. These include carcinoma, chronic inflammation, renal calculi, nephritis, cystitis, and hemorrhagic diseases. In some cases, hematuria is the only reliable clinical symptom to aid the physician in diagnosis and treatment. You are probably already aware that hematuria occurs when incompatible blood is transfused into a patient. Trauma to the kidneys from an injury also produces bloody urine. In general, we can state that a significant amount of blood in the urine is a serious pathological finding unless it is the result of menstrual discharge. It is for the physician to determine how much blood is significant; however, a normal 24-hour urine specimen may contain up to 650,000 RBC's. This would represent only an occasional RBC per low power field (i.e., one cell seen in 3 to 6 lpf). This value is the same for males and females but the possibility of contamination from the vagina or uterus of females is considerable. Urine which contains gross blood (discernible macroscopically) should be positive for protein.

8-14. The appearance of erythrocytes (RBC's) varies considerably depending upon the pH, specific gravity, and age of the specimen. Red cells may sometimes be confused with yeast cells or white blood cells. Actually, white blood cells are one and one-half times as large as red cells, and yeast cells frequently show budding. If there is any doubt, add a drop of 10 percent acetic acid. Red blood cells will hemolyze, whereas other structures are unaffected. As mentioned in a preceding paragraph, the benzidine test will detect as few as 4 or 5 red blood cells per high



INTACT RED BLOOD CELLS

'GHOST' BLOOD CELLS



CRENATED RED BLOOD CELLS

Figure 9. Three types of RBC's.

power field. Red cells may be intact, crenated, or ghost (hemolyzed). All three types are shown in figure 9.

8-15. Crenation of erythrocytes occurs in *hypertonic* urine, and hemolysis is due to swelling and bursting of the red cells in *hypotonic* urine. As you will recall from your study of clinical chemistry, a hypertonic solution is one in which the concentration of ions (other than water) is greater outside the cell than inside. A hypotonic solution is a solution in which the concentration of ions is less outside the cell than inside. Consequently, in a hypertonic medium water is withdrawn from the cells, and in a hypotonic medium water moves into the cell. Dilute urine is hypotonic, and as a result, *ghost* forms of erythrocytes may be observed. The urine in this case will still give a positive result in chemical tests which detect hemoglobin. Large amounts of blood that have been present in urine for any length of time will cause the color of urine to be a smoky red-brown. However, bile or various dye substances which are excreted may also cause the urine to appear this color. Hence, color alone is not a reliable indication of blood. Fresh blood in urine appears bright red.

8-16. **Casts.** Cylindrical bodies which are formed in the lumen of the renal tubules are referred to as *casts.* They usually consist of a *hyaline* matrix in which organized sediment is imbedded.[4] However, other types of casts may also be found on occasion. Casts are differentiated by their microscopic appearance (coarse granules, fine granules, hyaline); identification of inclusions (epithelial, red cell, bacterial); the chemical nature of their inclusions (waxy, fatty, hyalin); and finally by their histochemical reactions or staining characteristics. These are overlapping distinctions, and you must keep this in mind as they are discussed.

8-17. Table 1 represents one classification of casts in urine. Let us discuss each of the types of casts mentioned in this table. Note that our system of classification does not follow that outlined in AFM 160–49, *Laboratory Procedures in Clinical Chemistry and Urinalysis.* The classification presented in this chapter is somewhat more systematic and based upon distinctions which can be discerned with the Sternheimer-Malbin stain.

8-18. If you will look at the listing in table 1 you will see two major categories of casts: A, Hyaline, and B, Epithelial. These distinctions are based upon the original material which

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[4] Hyaline casts. The term *hyalin* (hi' ah-lin) is often confused with *hyaline* (hi' ah-lin), and in fact these terms are sometimes used synonymously. Technically, *hyaline* refers to an optical characteristic, and implies transparency. *Hyalin* is a translucent albuminoid substance.

TABLE 1
CLASSIFICATION OF CASTS IN URINE

A. Hyaline
    1. Transudation (clear hyaline)
    2. Inclusion Casts
        a. Fine Granular
        b. Coarse Granular
        c. Tubular Epithelial
        d. Red Cell
        e. Other -- Bacterial, Crystalline, Fatty

B. Epithelial
    1. Fresh Cell Cast
    2. Degenerated
        a. Fine Granular
        b. Coarse Granular
        c. Waxy
        d. Fatty

formed the cast. The *clear hyaline cast* is formed by the precipitation of protein in the lumen of the kidney tubules. Hyaline casts rapidly disappear in an alkaline medium. A hyaline cast consists exclusively of a colorless, slightly refractile matrix. Consequently, it has the appearance of almost complete transparency. If stained, it appears pale pink to light purple and has a homogenous central portion.[8] Hyaline casts are sometimes called transudation casts. A clear hyaline cast is illustrated in detail C of foldout 1.

8-19. In addition to clear hyaline casts there are various types of inclusion casts under the broad classification of hyaline (see table 1). Practically any cellular material which is present in the kidney tubules may be trapped in the precipitated matrix of a hyaline cast. The casts may appear finely or coarsely granular. Granules stain purple, and the hyalin matrix stains pink. The cellular source of the granules is uncertain. They may be degenerated blood cells or epithelial cells. If the tubular epithelial cells are intact within the hyalin matrix, we refer to the cast as a *tubular epithelial inclusion* cast. The epithelial cells are from the renal tubules and represent a pathological process. A *tubular epithelial* cell inclusion cast is shown in detail D of foldout 1.

8-20. Refer again to table 1. Under A,2, *hyaline inclusion* is the red cell inclusion cast. Unstained red blood cell casts are usually orange in color. If the cast appears homogenous without defined cellular outlines, it is referred to as a *blood cast* rather than a *red cell cast*. Some evidence suggests that clinical value can be derived from identifying these two kinds of casts. Blood casts are rarely seen unless associated with glomerulitis or, in some cases, with collagenic disorders. Red blood cell casts occur with greater frequency in many other conditions. A red cell inclusion cast appears as a mass of red blood cells which stain pale lavender. The cells are usually tightly packed, so that little of the pink staining matrix can be seen. Graduations of the two types may be found. Both the blood cast and the red cell cast are compared in detail E of foldout 1.

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[8] For the remainder of this chapter, "stained sediment" will mean sediment stained by the Sternheimer-Malbin technique.

8-21. The final listing in table 1 under hyaline inclusion cast includes bacterial, crystalline, and fatty hyaline inclusion casts. Bacteria within casts stain dark purple and can usually be identified as bacteria without difficulty. Occasionally bacteria may be seen within the white blood cells, giving rise to *glitter cell casts.* Crystals can be identified within casts by their characteristic geometric design. Ordinarily, it is not necessary to identify the cast beyond the fact that it is a *crystal* inclusion cast. A fatty inclusion cast incorporates highly refractive fat droplets. Fat will not stain with the Sternheimer stain. Fatty casts are characteristic of degenerative tubular disease.

8-22. The second major classification of casts in table 1 is B, *Epithelial.* Epithelial casts, in contrast to hyaline casts, are formed by desquamated epithelial cells which are not cemented by a hyalin matrix. Rows of cells may slough off the kidney tubules, suggesting considerable damage to the tubule lining. The mold of the kidney tubule, itself, shapes the cast. If this type of cast appears with distinct cell margins, it may be referred to as a *fresh tubular epithelial cell cast* (see detail D of foldout 1). However, an epithelial cell cast of this type does not always remain intact. It may degenerate to form a *coarse granular cast or a fine granular cast.* These two kinds of casts are illustrated in detail F of foldout 1. There is no hyalin material present which can be detected with the Sternheimer-Malbin stain. If the cast disintegrates further until a homogenous mass is formed, it then becomes a *waxy cast.* Detail F of foldout 1 also shows a waxy cast. A waxy cast is the most degenerate form of an epithelial cell cast. It stains a uniformly dark purple. This is due to the inclusion of amyloid, an abnormal protein which deposits in the kidney.[6] The waxy cast is the most likely to be found in urine of high specific gravity following a period of oliguria. Waxy casts are found in cases of severe renal lesions and in some cases in amyloid degeneration and advanced nephritis.

8-23. The size of casts is a consideration worth noting. Of course, a cast from a child will be smaller than a cast from an adult because the lumina of the tubules in a child's kidney are smaller. With due regard to this expected difference, casts are occasionally seen which are very narrow. This is significant because it suggests a swollen tubular epithelium. Conversely, very broad casts may be observed. Their presence is an important laboratory finding. *Broad casts* are formed in the large collecting tubules and result from urinary stasis which, in turn, is caused by the diminished function of large numbers of nephron units. Although they are usually granular, broad casts may be any of the types previously discussed and are associated with renal failure.

8-24. It is important to recognize that cast formation is generally described in terms of theories. Consequently, it is difficult to classify casts without subscribing to a particular theory of how casts are formed. One theory suggests that waxy casts and fatty casts are derived from hyaline casts; and that epithelial casts are merely one type of hyaline cast. More recently, some authorities have identified two types of epithelial and granular casts, one being hyaline, and the other nonhyaline. This distinction was made in the preceding discussion because there is a type of granular cast in which a hyalin (albuminoid) matrix cannot be demonstrated. It is assumed that this later type of granular cast is derived from epithelial casts which have either lost their hyalin matrix or never contained a hyalin matrix. These casts may contain protein, but not the hyalin material which is characteristic of hyaline casts. The need to distinguish the two categories of granular casts (i.e., granular hyaline inclusion and granular desquamation casts) is still uncertain. Actually, the distinction is easy enough to make based on the respective histochemical reactions with the Sternheimer-Malbin stain.

Figure 10. Cylindroids.

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[6] Amyloid. An *amyloid* is a starch-like protein, probably a glycoprotein. The exact chemical composition of amyloid has not been determined.

Figure 11. Yeast cells.

Hyalin stains bright pink. Epithelial cell casts without a hyalin matrix are desquamation casts. Epithelial casts with a hyalin matrix are inclusion casts. This distinction may be of some value to the clinician.

8-25. *Cylindroids* resemble casts, but usually have one drawn-out tapered end. They are of no great clinical significance other than being present in increased numbers in inflammatory conditions. Their origin is not known. Mucus threads are long, slender, transparent strands which may normally be found in small numbers. Increased numbers are present in urethra and bladder irritations. They are usually twisted into various shapes with a tapered end as shown in figure 10, and this characteristic aids in distinguishing them from casts.

8-26. Spermatzoa are easily identified by their characteristic shape, but their presence is generally not reported unless specifically requested by the physician. Yeast cells may be present pathologically or as contaminants. These cells are sometimes confused with red cells. A careful study of the specimen will usually reveal budding which is a characteristic of yeast cells as suggested in paragraph 8-14 and illustrated in figure 11. Parasites are sometimes found in urine, too. The most common are *Trichomonas* species. *Shistosoma haemotobium* is less common but may be found in the urine. A discussion of these parasites will be found in Career Development Course 90412.

9. Crystalline and Amorphous Sediments

9-1. The majority of the crystals found in fresh urine are not clinically significant. If present in greatly increased numbers, however, they may be important. In addition, certain pathologic conditions are accompanied by the excretion of abnormal crystals in the urine. Learn to identify the common types and the most common clinically significant crystals. In doubtful cases it may be advisable to consult the physician and inquire about medication and other pertinent facts which may have produced the crystals in question.

9-2. An approximate classification of inorganized sediments based upon the pH of the urine may be used, but this division is not absolute. For instance, some crystals normally found in acid urine may be present in a sample which is neutral or slightly alkaline. The change from acid to alkaline urine crystals occurs gradually as the acid constituents deteriorate. Upon aging of the specimen, the number of crystals increases. Since most inorganic substances are more soluble at higher temperatures, cooling of urine will cause precipitation of crystals. In some cases it may be necessary to gently warm specimens taken from the refrigerator or change the pH in order to obtain a clear microscopic field. Heavy amorphous sediment (without observable structure) will make it very difficult to accurately perform a microscopic analysis.

9-3. **"Normal" Crystal and Amorphous Content of Alkaline Urine.** Triple phosphate (ammonium magnesium phosphate), dicalcium phosphate, amorphous phosphates, and ammonium biurate crystals are frequently found in alkaline specimens. These crystals are all soluble in acetic acid and as a group, may be differentiated from other crystals by their characteristic.

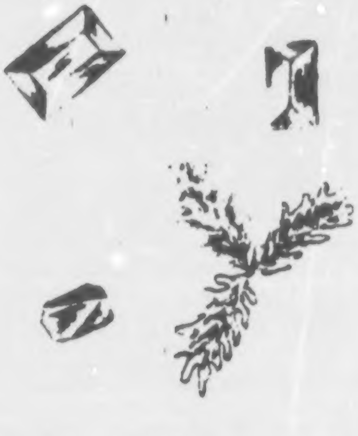

Figure 12. Triple phosphate crystals.

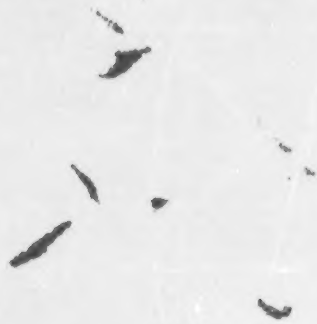

Figure 13. Ammonium biurates.

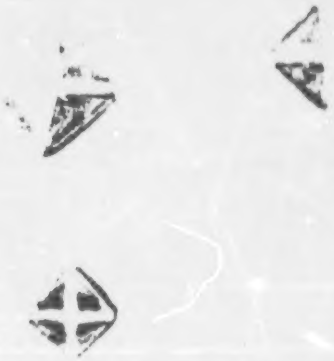

Figure 15. Calcium oxalate.

9-4. Triple phosphate crystals are colorless, high refractile prisms varying in size and presenting three, four, or six sides, giving the typical coffin-lid forms. The edges will often appear colored. Dicalcium phosphate usually crystallizes near the neutral point (pH 7.0), forming slender, colorless prisms with one pointed end. Various forms of triple phosphate crystals are given in figure 12. Amorphous phosphates are common in alkaline urine and appear as a granular precipitate.

9-5. Ammonium biurates are precipitated only when free ammonia is present. They may be found in several different forms, for example, in sheaves of fine needles and dumbbells as pictured in figure 13. They have a characteristic yellow color, and dissolve when the urine is warmed. Ammonia is liberated upon the addition of acetic acid.

9-6. **"Normal" Crystal and Amorphous Content of Acid Urine.** Uric acid, amorphous urates, and calcium oxalate crystals are often found in acid specimens, particularly after standing. Uric acid crystals are found in many different forms. The rhombic shape with an occasional rosette, as seen in figure 14, is typical. Uric acid crystals are soluble in sodium hydroxide. They are typically yellow or red-brown in color and may, like urates, give a cloudy or milky appearance to the specimen.

9-7. Amorphous urates are common and appear as a granular precipitate, usually pigmented. Differentiation between this type of urates and amorphous phosphates may be made by noting the pH of the urine, the effect of 10 percent sodium hydroxide, or gentle heating of the urine.

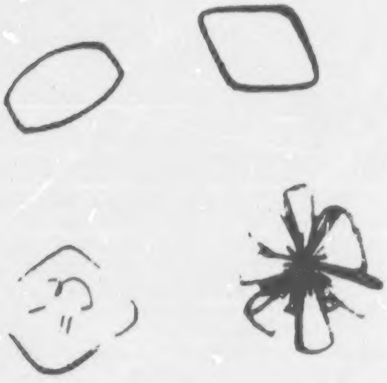

Figure 14. Uric acid.

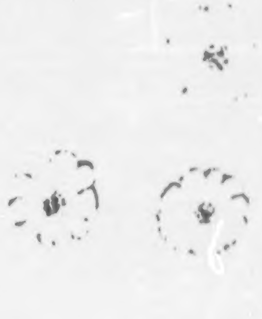

Figure 16. Leucine.

Figure 17. Tyrosine.

Amorphous urates are soluble in alkali and upon warming.

9-8. Calcium oxalate crystals are most frequently found in acid urine but may also appear in neutral and alkaline urine. They are of little importance, and usually result from a rich diet of tomatoes, rhubarb, and asparagus. Calcium oxalate crystals are soluble in hydrochloric acid but not in acetic acid. Calcium oxalate crystals vary greatly in size and shape but are generally seen as colorless, octahedral (box-shaped) crystals resembling small squares crossed by two intersecting diagonal lines ("envelope" appearance) as pictured in figure 15. They may also appear as dumbbells or spheres.

9-9. **Abnormal Crystals.** It is possible that you will find crystals of some of the amino acids or crystals of cholesterol in urine. The three amino acids we will consider are leucine, tyrosine, and cystine.

Figure 18. Cystine.

9-10. Leucine and tyrosine crystals are cleavage products of protein and usually occur simultaneously. They are formed as a result of serious liver damage, but they are rarely found in urine. Leucine crystals are yellow spheres often possessing radial and concentric striations, as illustrated in figure 16. They are soluble in alkali, but not in dilute hydrochloric acid or in dilute acetic acid at room temperature. Tyrosine crystals appear as very fine needles usually arranged in sheaves with a constriction in the middle as sketched in figure 17. Tyrosine crystals show the same solubility pattern just described for leucine, except tyrosine is not soluble in boiling acetic acid, whereas leucine dissolves in this solvent.

9-11. There are rather simple chemical tests for tyrosine and leucine. To test for tyrosine, adjust the pH of an aliquot of the urine to be tested to 5.8. Then place the sample in the refrigerator until crystals are formed and can be separated. To this crystalline precipitate, add 2 ml. of Morner reagent.[7] The mixture is heated to boiling and observed for the appearance of a green color which is considered a positive test for tyrosine.

Figure 19. Cholesterol.

9-12. To test for leucine, adjust the pH of an aliquot of urine to 6.8 or 7. Allow crystals to precipitate in the refrigerator. Then dissolve the precipitate in a few milliliters of water and add 1 drop of 10 percent copper sulfate. Leucine gives a blue color which remains stable when the mixture is heated.

9-13. Cystine is also a breakdown product of protein that appears very rarely. The crystals occur in acid urine as colorless, highly refractile, hexagonal plates with well defined edges as shown in figure 18. Cystine crystals are not soluble in acetic acid; however, they are soluble in hydrochloric acid and in alkali. Crystals that have been separated may be identified by the Sullivan test. In this test, crystals are dissolved in 2 ml. of water to which a few drops of sodium hydroxide have been added. Add 1 ml. of 5 percent sodium cyanide solution and allow the mixture to stand for 10 minutes. In rapid succession, add the following: 0.5 ml. freshly prepared 0.5 percent 1, 2 napthoquinone-4-sodium sulfonate and 2.5 ml. 10 percent anhydrous sodium sulfite in 0.2*N* sodium sulfite. After 30 minutes add 0.5 ml. 2 percent sodium hyposulfite in 0.2*N* sodium hydroxide. The appearance of a red color is positive for cystine.

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[7] Morner reagent. 1 ml. formalin, 45 ml. water, and 55 ml. sulfuric acid added in this order.

9-14. Cholesterol crystals are rarely found. They have a characteristic "missing corner" and appear in acid specimens as large, flat, colorless plates as sketched in figure 19. Other rarely found crystals include calcium sulfate, hippuric acid, hematoidin, indigo, and fatty acids.

9-15. **Sulfonamides.**[a] Following treatment of the patient with sulfa drugs, crystals of the sulfonamides and their derivatives may be found in either alkaline or acid urine. Conjugated derivatives, e.g., acetylsulfathiazole, are less likely to be found in alkaline urine. Sulfamerazine and sulfadiazine are also less likely to be found in alkaline urine because of their increased solubility at higher pH levels. Unfortunately, the crystals which you will observe are often derivatives of the drug that was administered. Consequently, the shape of the crystals will vary considerably. Free sulfonamides and acetylsulfanilamide are less likely to be found than other derivatives because of their relatively high solubility. Rather than attempt to identify all of the various sulfa crystals, consult the physician concerned and ascertain the importance of the identification. If it is important to investigate, a book such as *Bray's Clinical Laboratory Methods* or AFM 160–49 will be helpful.

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[a] **Sulfonamides.** This is a group of compounds consisting of amides of sulfanilic acid which have in recent years replaced the more toxic parent compound, sulfanilamide (a product of coal tar) for the treatment of bacterial infections.

CHAPTER 5

Chemical Examination

CHAPTER 2 OF this volume related the basic physiology of the renal system. You may wish to refer to Chapter 2 as we begin a description of qualitative urine chemical tests. You should recall that the quantitation of some urinary chemical constituents and their physiological significance have previously been studied in Volumes 2 and 3 of this course. In these preceding volumes on blood chemistry where the chemical constituent was common to both blood and urine, the quantitative aspects of urine chemistry were also discussed. For example, the chapter on proteins of Volume 2 covered chemical and metabolic aspects of protein excretion. The physiological chemistry of liver function also included consideration of bile pigments and the physiological mechanism by which they appear in urine. Kidney function tests were covered at length in Volume 3.

2. This chapter will deal with the technical aspects of screening urine specimens (qualitative analysis) for various biochemicals of clinical importance and, when necessary, roughly estimating the quantity of a chemical constituent (semiquantitative analysis). As you study this chapter, keep in mind that although the methods used are the simplest in the clinical laboratory, the physiological mechanisms which you are measuring involve very complex biochemical principles.

10. Urinary Protein

10-1. A qualitative test for protein in urine (proteinuria) is one screening test always included in routine urinalysis. Healthy adults normally excrete less than 100 mg. of protein in 24 hours. This protein excreted by the kidneys has been shown to be derived from the plasma proteins. Although albumin is the primary protein fraction normally excreted, smaller quantities of globulin are also eliminated. It is theorized that more albumin is excreted by the kidneys because of its relatively low molecular weight (70,000) and correspondingly smaller molecule. Normally, the glomerular filter is impermeable to protein molecules larger than albumin, e.g., globulin (MW 165,000).

10-2. In active renal disease, protein-like substances called proteoses have been identified in urine by electrophoresis. Proteoses migrate with the globulins electrophoretically and interfere with some tests for protein. Protein is also contributed to urine from the lower genitourinary tract. This includes protein from the ureters, bladder, urethra, and prostate. These constituents are nonserum proteins with an electrophoretic mobility slightly greater than albumin. They are the so-called "Tamm-Horsfall" mucoproteins and are important because of their confusion with serum-derived protein.

10-3. Transitory proteinuria may result from extraordinary physical exercise or ingestion of a high protein meal. The temporary impairment of renal circulation which results from postural aberration will also cause a transitory proteinuria. This is known as orthostatic proteinuria, and it disappears when the impairment is relieved. Proteinuria in these cases cannot be attributed directly to kidney disease. It is usually temporary and does not exceed 500 mg./day. Proteinuria may also occur in disease states that are not primarily renal, such as febrile conditions. Another extrarenal cause of albuminuria is bleeding into the urogenital tract as a result of trauma or disease. This could result from trauma during the passage of urinary calculi, for example.

10-4. The critical importance of testing for proteinuria, however, is in the diagnosis of kidney disorders. It has been said that proteinuria is probably the best single indication of renal disease. For this reason, proteinuria is always throughly evaluated by an attending physician. Consequently, most authorities recommend that all positive urine protein screening tests be confirmed by a second, different qualitative test. Confirmatory testing in this instance is done for two reasons: (1) the clinical importance of the result and (2) the limitations of any single method.

10-5. **Urine Protein Methods.** Probably because of the long history of urinalysis and its time-honored place in clinical diagnosis, many methods exist for the qualitative analysis of proteinuria. Although older texts and some newer revisions refer to *albuminuria*, it is a misnomer. All qualitative analyses used in urinalysis identify protein and are not specific tests for the albumin fraction. Some reagents used in testing for proteinuria are more selective than others. You may recall that in Volume 2 of this course we spoke of various precipitants for protein and explained the "salting out" of specific protein fractions. You should remember also that these reactions were dependent upon the nature of the protein (i.e., isoelectric point) and the nature of the reagents (i.e., pH and salt concentration). These principles are also important to remember in studying the qualitative analysis of urinary proteins.

10-6. A complete listing of all the known urine protein screening tests is impractical. These tests involve the use of heat and acetic acid, sulfosalicylic acid, trichloroacetic acid, picric acid, nitric acid, and biuret reagent (used alone or in combination with salts such as sodium chloride, magnesium sulfate, potassium ferrocyanide, and sodium acetate). The literature is filled with these methods and more. Some have been used as salts in spot tests, in dilute and concentrated solutions, and as test tube ring tests.

10-7. The more modern approach is to use a buffer salt with an indicator which changes color at different protein concentrations. Such a reagent has been incorporated into a well-known test strip for rapid screening of urine specimens. This procedure will be discussed in more detail later in this chapter. Because of the necessity for a confirmatory test, several other methods will also be included in our discussion. The heat with acetic acid technique and sulfosalicylic acid test are probably the most widely used of these other tests.

10-8. **Prerequisites.** At this point we might consider what prerequisites are desirable in a qualitative screening test for urine protein. First of all, it must detect *abnormal* amounts of protein. In order to define an abnormal amount, we must first decide upon the so-called "normal" limits. Since we are concerned with a *screening* test, we will be interested primarily in only the upper normal limit. Ranges in the literature for normal 24-hour urine protein extend from a low of 20 mg./24 hours to a high of 250 mg./24 hours. This wide range is understandable in view of variables which influence this value, such as 24-hour urine volume, diet, method of analysis, and normal individual physiological fluctuations. It is obvious that clinical interpretation by a physician is essential to determine any specific norm for a particular individual.

10-9. Considering all the above factors, current literature has establisehd an upper limit of "normal" between 70 and 100 mg./24 hours. If normal fluctuations in urine volume are accounted for, this amounts to 7 to 10 mg./100 ml. of urine. Since the method employed is important, it should be noted that these analyses used trichloroacetic acid precipitation in two cases with the biuret color reaction for the lower value (20 to 75 mg./24 hours) and photoelectric measurement of turbidity for the higher value (20 to 100 mg./24 hours). Precipitation with alcohol and reaction with biuret yielded the lowest value of "at least 71 mg./24 hours." It was demonstrated electrophoretically that alcohol does not precipitate most of the proteoses.

10-10. These figures establish another criterion for a qualitative urine protein test. The test should not be so sensitive that it will react with "normal" amounts of urine protein. In other words, the sensitivity should be no greater than 5 to 10 mg./100 ml. of urine. Under certain conditions, trichloroacetic acid and sulfosalicylic acid will detect protein concentrations of less than 1 mg./100 ml. Consequently, under such conditions, these tests would be unacceptable for screening urine protein. The problem of excessive sensitiviy can be eliminated with appropriate dilution of the protein specimen. The biuret reaction which you studied in Volume 2 is also extremely sensitive to dilute protein solutions. The difficulty in using biuret as a screening test arises from its nonspecific reactions with many other normal urine constitutents.

10-11. **False Reactions.** False reactions have always been a problem with urine screening tests. As stated previously, erroneous reactions are the prime reason for confirmatory testing. If you will look at table 2, you will see some of the possible causes for false reactions reported in the more common protein screening tests. It is evident from the table that the nitric acid ring test reacts with many substances found in normal urine. The excessive number of erroneous reactions renders this test practically worthless as a protein screening test. The Roberts[*] modification is said to have fewer nonspecific reactions, but research support for this premise is lacking.

10-12. It should also be stated that the false reactions listed are not invariable. For instance, penicillin "in massive doses" is reported to interfere with both the nitric acid and sulfosalicylic

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[*] Roberts Reagent. 1 part concentrated nitric acid added to 5 parts saturated aqueous magnesium sulfate.

TABLE 2
POSSIBLE FALSE REACTIONS REPORTED IN QUALITATIVE URINE PROTEIN TESTS

Causable Agent	Sulfosalicylic Acid (Exton's Reagent)	Nitric Acid (Heller)	Heat & Acetic	Heat & Buffered Acetic	Reagent Strip
Urine Turbidity[1]	±	±	±	±	
Tolbutamide[2] Metabolites	+	+	+		
X-ray Media Iodine	+	+	+		
Penicillin	+	+	+		
Sulfa	+	+	+		
PAS[3]	+	+	+		
Strong Alk. Buffer	-		-		+
Proteoses	+	+	+	+	
Mucin		+			
Resinous Acids[4]		+	+	+	
Urea		+			
Uric Acid	+	+			
Strong Dye					±
Reagent Contamination					±
Leaching Reagent					-

1. May be removed by centrifugation
2. Used in treatment of diabetics
3. Para-amino-salicylic Acid
4. From drugs such as benzoin

TABLE 3

POSSIBLE REACTIONS OF URINE AND ACETIC ACID IN THE QUALITATIVE SCREENING TEST FOR PROTEIN

Specimen Appearance	After Boiling	After Acidifying	After Reboiling	Interpretation
Clear	Clear	Clear		Normal*
Clear	Cloudy	Clear		Phosphates
Clear	Clear or Cloudy	Clear Effervesces		Carbonates
Clear or Cloudy	Below 60°C. Cloudy	Cloudy	Over 60°C. Clear	Bence-Jones or Proteoses
Clear	Cloudy	Sl. Cloudy to Flocculent	Same or Increase Cloud	Positive** Protein
Cloudy	Clear	Clear		Urates
Cloudy	Clear	Clear	More Cloudy	Excess Urates
Cloudy	Cloudy	Cloudy	Cloudy	Microorganisms*

* Normal Protein Less Than 5 to 10 mg/100 ml.
** Positive Protein Greater Than 5 to 10 mg/100 ml.
***Microorganisms Confirmed Microscopically

acid tests with no qualification as to a more exact concentration. Further qualification in the legend of table 2 should be noted. As it indicates, urine turbidity can be discounted entirely as the cause of erroneous results if the urine can be cleared by centrifugation. You are aware that clearing by centrifugation can be done with many specimens, and that centrifugation is the usual procedure when a microscopic examination is requested.

10-13. If proteoses are interfering, they can be dissolved in saturated picric acid, which also precipitates protein. Thus, in this case a subsequent test specific for the interfering substance may also be used to substantiate results. Modifications in method, such as the use of heat with sulfosalicylic acid, can be used to dissolve proteoses and Bence-Jones protein without affecting precipitated albumin and globulin. These dissolved substances can be identified by clearing of the specimen when it is warmed and thereby excluded as a cause of false reactions.

10-14. Subjective interpretation by the technician, although undesirable technically, is unavoidable with these qualitative tests. In table 3 the listing of reactions observed with heat and acetic acid at various points in the procedure clearly illustrates the interpretation required of a technician. Without a thorough understanding of these reactions, valid results are not attainable.

10-15. Even the widely used reagent strips are subject to false reactions. Highly buffered alkaline urine which may be encountered in aged specimens gives a falsely positive color reaction. This reaction is noted in table 2. The reason is that the strip contains a buffer salt which maintains a pH of 3 and the indicator will indicate protein concentration accurately, only at this pH. Any substance which breaks this buffer system will invalidate the reaction. This principle is detailed in paragraph 10-22. If the buffer salt deteriorates through improper storage or is contaminated in handling, the buffer system could be destroyed. Any strongly alkaline reaction with a positive protein strip test should be checked with heat and acetic acid.

10-16. As shown in table 2, the heat and acetic acid test may give a false *negative* reaction in highly buffered alkaline urine. If it is *positive*, however, it is a valid positive protein test. A false reaction is less likely to occur if the acetic acid contains buffer salts (see paragraph 10-20). One last factor which involves only the reagent strip test for protein is the matter of highly colored urine. Such specimens are occasionally encountered, and since the reacted strip presents a color reaction, strong chromogens may confuse this color differentiation. The range of colors for a positive protein reaction are on the Labstix indicator in the training aids package.

10-17. It must be obvious at this point that no single qualitative test for protein is perfect. Let us review the prerequisites for a qualitative urine protein screening test. First, it should be sensitive enough to detect all abnormally high concentrations of urine protein, but not so sensitive that it reacts with normal urine protein concentrations. Second, it should have few, if any, false positive or negative reactions. However, since no protein screening test is without false reactions, you as a technician should be aware of the reactions that may occur. Then you should know what alternate screening test will verify the true protein reaction. The stability of reagents used for the test should be included among the prerequisites. Finally, the number of urine specimens processed in most laboratories demands an efficient operation, and the medical staff naturally insists on technical competence. You must select the screening test or tests which are both efficient and appropriate.

10-18. **Heat and Acetic Acid.** Heat and acetic acid has been mentioned several times as a screening test for urinary protein. The principle of the test is simply that urinary protein will coagulate when heated at an optimal acid pH. Several modifications have been used. The method used for the reactions obtained in table 3 began with 2 to 3 ml. of unfiltered urine. The urine was first boiled thoroughly then acidified with 2 to 3 drops of 50 percent (V/V) acetic acid and boiled again. The readings were observed as they are indicated in table 3.

10-19. A modification of the heat and acetic acid method uses 10 to 15 ml. of centrifuged, clear urine in a pyrex test tube. Centrifugation removes most crystals and cellular components which give a cloudy appearance. After clearing, the upper portion of the specimen is heated to boiling. At this point, if the upper portion remains clear, the urine is negative for protein. If it is cloudy, it is acidified with 2 to 3 drops of glacial acetic acid and reboiled. A persistent cloud after reboiling is a positive reaction of protein. Study the list of reactions again in table 3 to be sure you understand the sequence of reactions which represent both positive and false reactions with this method.

10-20. The purpose of boiling the specimen is to drive off carbon dioxide (CO_2), lowering the buffer capacity, which in turn will make the normally acid urine even more acid. The optimal pH for protein precipitation is between 4 and 5. However, you will notice in table 3 that phosphates and carbonates as well as protein may precipitate after this initial boiling. Therefore, acetic acid is added to dissolve phosphates and react with carbonates, which go off as gas, leaving protein and perhaps excess urates. You can imagine that a highly buffered, alkaline urine may prevent the urine from becoming acid enough to precipitate protein. This results in a false negative reaction for urine protein.

10-21. More recently it has been proposed that sodium acetate be added to the acetic acid to form an acid reagent buffered at pH 4. This reagent would tend to stabilize the reaction at an optimal pH for protein precipitation. As you can see in table 2, however, proteoses and resinous acids (from drugs) will react even in the buffered acid reagent. Although sulfa-para-amino salicylic acid, and high concentrations of penicillin have been reported to interfere with the heat and acetic acid without buffer, they do not apparently cause false positive reactions with the buffered acid reagent.

10-22. **Reagent Strips.** The reagent strip test for protein is based upon a rather time-honored principle. In 1909, Sorensen noticed that certain pH indicators turn different colors at the same pH in the presence of protein. He called this phenomenon the "protein error of indicators." In 1957, this principle was used commercially to prepare a powder mixture of bromphenol blue indicator and a salicylate buffer (pH 3) in tablet form. In tests with urine specimens positive for protein, it was discovered that the concentration of protein in the specimen affects the intensity of the blue-green positive reaction. Subsequently, an absorbent paper strip was impregnated with tetrabromphenol blue indicator and a citrate buffer at pH 3. The negative indicator color is yellow with a range of positive colors from light green to aqua blue. These colors are illustrated in the Labstix indicator of the training aids package.

10-23. The Labstix strip is a later development available from standard military supply sources. A plastic strip with attached porous paper squares is used to facilitate drainage when the reagent strip is dipped in urine. The nonwettable plastic prevents an excess of specimen from being absorbed by the reagent squares. As we stated previously in Chapter 1 of this volume, the reagent strip must not be dropped into the specimen. Follow the directions as they are given on the reagent bottle. These directions are on the Labstix indicator.

10-24. You will notice that the time of reading is not critical for the protein portion of the reagent strip. Relative concentrations of protein in mg./100 ml. are shown on the protein color guide. However, critical quantitative analyses have demonstrated that qualitative tests are poor indicators of the actual protein content. To avoid confusion, it is suggested that the protein concentration shown on the color chart *not* be used. Preferably, a relative concentration report of trace, 1+, 2+, 3+, and 4+ should be made for this screening test.

10-25. The pH indicator portion of the reagent strip is immediately adjacent to the protein indicator square. In view of possible false positive reactions with strongly alkaline urine, you should consider the pH and protein portions together when you read a protein reaction. A pH above 8 with a positive protein should be reevaluated with another test for protein, i.e., heat and acetic acid.

11. Urine Glucose

11-1. As we stated in describing tests for proteinuria, tests for glucose in urine (glucosuria) are also determined in all routine urinalyses. Screening urine specimens for glucose is the best method of detecting diabetes mellitus. It is also of critical importance to the physician in his treatment of the diabetic patient. When you consider that diabetes afflicts approximately 5,000 persons per million of population, the importance of detection and treatment becomes even more apparent. Often glucosuria does not occur until the blood glucose is considerably elevated. How high the blood glucose level will rise before glucosuria occurs depends upon the renal threshold of each individual. In physiology, renal threshold is defined as that concentration of a substance in the blood plasma above which the substance is excreted by the kidneys and below which it is not excreted.

11-2. You should know, however, that diabetes is not the only cause of glucosuria. Kidney disease which affects the reabsorptive capacity for glucose of the renal tubules will also produce glucosuria. Pancreatic disease, endocrine disorders, and damage to the central nervous system may cause glucose excretion in urine. It may also be found during stress situations and pregnancy, or associated with anesthesia. Another sugar, lactose, is detected more often in pregnant women, and it increases after delivery during lactation. Lactose is important because of its positive reaction in nonspecific tests for glucosuria.

11-3. Glucose, lactose, galactose, fructose, and pentoses (rhamnose, arabinose, and xylose) are all sugars which may be excreted in urine. They are called reducing sugars because of their chemical reaction in several nonspecific urine sugar tests, notably Benedict's reaction. This will be explained in more detail later in this section. The

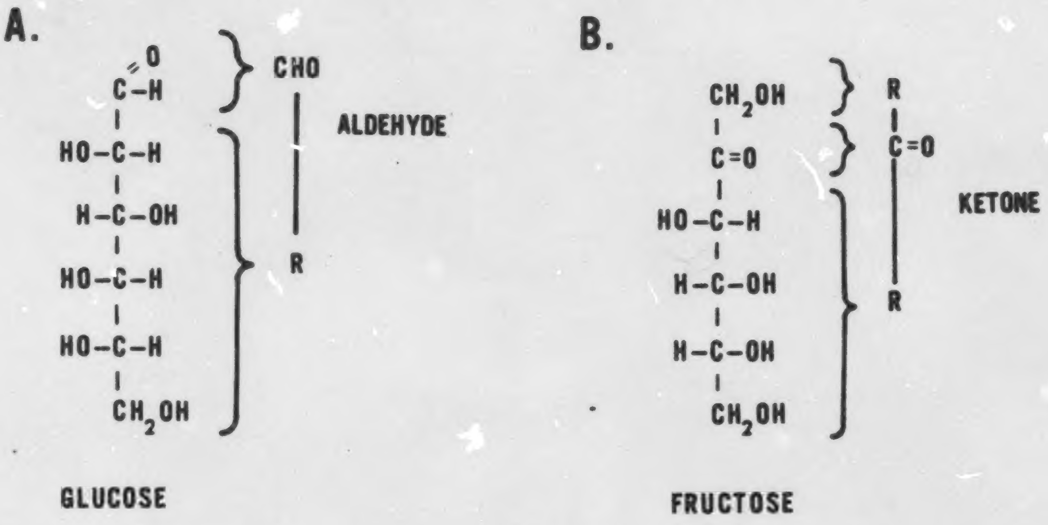

Figure 20. Relationship between the general formulas for (A) aldehyde with glucose and (B) keto- with fructose.

occurrence of glucosuria and lactosuria has been discussed. Galactosuria, fructosuria, and pentosuria are found in the urine of individuals with inherited metabolic defects. In addition, pentoses may be excreted after eating large amounts of fruits such as plums and cherries. Positive findings with the galactose and xylose tolerance tests (in which these sugars are ingested) will cause galactosuria and pentosuria. Reducing sugar tests on these urine specimens will be positive. Tests which are more specific (i.e., glucose oxidase) are required to rule out the presence of glucose.

11-4. **Urine Reducing Substances.** For years the detection of glucose by its chemical reducing effect has been a routine procedure in urinalysis. Benedict's test is the classic method for this purpose. Benedict's qualitative reagent contains copper sulfate, sodium citrate, and sodium carbonate. In this alkaline solution, any compound which contains free aldehyde (R-CHO) or keto- (R-CO-R) groups will reduce the cupric ions (Cu^{++}) to cuprous ions (Cu^{+}) upon boiling.

11-5. In figure 20 you can see the relationship between the general formulas for aldehyde with glucose and keto- with fructose. A precipitate of cuprous oxide (Cu_2O) if formed in the reaction. The color varies from green to yellow to red, depending upon the amount of reducing substance. The quantity and speed of precipitation also depend upon the concentration of reducing substance. The reaction with reducing sugars may be written as follows:

$$2\,Cu^{++} + \begin{matrix}\text{Reducing}\\ \text{Sugar}\end{matrix} \xrightarrow[\text{Heat}]{\text{Alkali}} Cu_2^{+}O\downarrow \quad + \text{Oxidized Sugar}$$

11-6. A commercial test tablet for reducing sugars is more often used instead of Benedict's reagent. It is a standard military supply item (Urine Sugar Test Tablet, FSN 6505–149–0220). The tablet contains copper sulfate and sodium carbonate as in Benedict's reagent. However, in addition, it contains sodium hydroxide and citric acid. The dry tablet is less stable, chemically, than the original liquid Benedict's reagent. Instructions are given with each bottle of tablets which detail observations associated with deterioration of the reagents. If these instructions are followed the reagent tablet is considerabley more advantageous than the liquid reagent. When a reagent tablet is added to urine and diluted with water, heat is generated by the sodium hydroxide as it dissolves and reacts with citric acid. The chemical principle is essentially that of the Benedict reaction. However, fewer false positive reactions due to reducing substances other than sugars are reported. Differences between the reagent tablet and the Benedict reaction compiled from the literature are indicated in table 4. Where differences have not been reported, we can assume that the reagent tablet reaction is the same as the Benedict reaction.

11-7. The directions and color range for the urine sugar test tablet are in the training aids package. The approximate amount of reducing substance is indicated on the color chart. One

TABLE 4

POSSIBLE FALSE QUALITATIVE REACTIONS FOR GLUCOSE IN URINE

Urine Constituent	Benedict's Test	Reagent Test Tablet	Glucose Oxidase Test
Reducing Sugars Other Than Glucose			
Lactose	+	+	
Galactose	+	+	
Fructose	+	+	
Maltose	+	+	
Pentoses	+	+	
Drugs or Metabolites of:			
Ascorbic Acid (Vitamin C)	+	+	-
Antibiotics with Ascorbic Acid	+	+	-
Oxytetracycline			
Tetracycline			
Chloral Hydrate	+		
Dipyrone			-
Dextrins (Pastry)	+	NR	
Glucosamine	+		
Indican	+	NR	
Isoniazid	+		
Meralluride			-
Para-amino-salicylic Acid	+		
Penicillin (Mass. Doses)	+		
Rhubarb	+		
Salicylates	+		
Santonin	+	NR	
Streptomycin	+		
Sulfonamides	+	NR	
Preservatives			
Chloroform	+	NR	
Formaldehyde	+	NR	
Cleaning Contaminants			
Hydrogen Peroxide			+
Sodium Hypochlorite (Bleach)			+
Detergents			+
Other			
Creatinine	+		
Protein	+	NR	
Homogentisic Acid (Alcaptonuria)	+		
Uric Acid	+		

+ False Positive, - False Negative, NR No Report precaution should be noted: The reaction must be observed as it occurs so that you will not miss a pass-through reaction. When the amount of reducing substance is greater than 2 percent, the reaction will rapidly "pass-through" green, tan, and orange to a dark green-brown color. If this occurs, the result should be reported as over 2 percent without reference to the color chart.

11-8. You should become thoroughly familiar with the false reactions listed in table 4. Even though this list appears extensive, it should not be considered complete. Tetracycline and oxytetracycline[10] antibiotics were first blamed for false positive reducing tests for glucose. Subsequently, it was discovered that ascorbic acid (vitamin C) added as a stabilizer to these antibiotics was the actual cause. Comparable levels of ascorbic acid are used with iron therapy in anemias, in vitamin supplements and in processed foods to maintain an appetizing color. Up to 1 percent may be added to some candies to preserve their color. Although these sources of vitamin C are not mentioned specifically as the cause of false positive glucose (reducing) reactions, their ascorbic acid content should alert a wary technician. As you can see, table 4 may very well be expanded when more complete information is available.

11-9. **Glucose Oxidase Method.** The existence of so many false glucose reactions in copper reduction methods (Benedict's) left something to be desired for glucose screening. Modern advances in enzyme technology produced an apparent solution with the introduction of glucose oxidase. As you learned in your study of enzyme chemistry, an enzyme is specific in its reaction with a substrate. The general reaction of the enzyme glucose oxidase with glucose is as follows:

$$\beta\text{-}D\text{-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

From the earlier discussion of stereoisomerism and mutarotation of carbohydrates (Volume 2) you should realize that glucose has several chemical structures. This concept is of interest here because in the reaction given above the β (beta) form of *D*-glucose reacts, whereas the α (alpha) configuration is over 100 times *less* reactive. Anhydrous *D*-glucose (shelf reagent) is primarily in the latter form, while urine glucose consists of both forms in equilibrium.

11-10. An equilibrium between the alpha and beta forms of *D*-glucose occurs after about 30 minutes in aqueous solution. This information is useful when you wish to check the reaction of glucose oxidase reagent strips. For quality control a known solution of beta-*D*-glucose should be used to check this reaction periodically to insure valid results. A very reliable glucose solution readily available for this purpose is a freshly opened bottle of *Coca Cola*. Looking at the reaction again, you see that in the presence of glucose oxidase, glucose reacts with oxygen (O_2). This is atmospheric oxygen. It is provided by removing the reagent strip from the urine specimen *immediately* after dipping. Sufficient oxygen will not be available if the strip is left in the specimen.

11-11. Hydrogen peroxide (H_2O_2) produced in the previous reaction, oxidizes orthotolidine in the presence of peroxidase to form a blue color. This may be written:

$$H_2O_2 + \text{Orthotolidine} \xrightarrow{\text{peroxidase}} \underset{\text{(blue color)}}{\text{oxidized Orthotolidine}} + H_2O_2$$

As you can see in the Labstix indicator, a positive reaction is denoted by a violet or deep purple color while a negative reaction is pink. Definite concentrations should not be assigned to these color reactions. They are approximations at best.

11-12. A continuously impregnated reagent strip is also available from Federal supply sources. It is used only for urine glucose. This strip is known as Tes-Tape. The indicator is included in the training aids package. You will notice that the color reactions are different from the multireagent strip mentioned above. With this reagent strip a negative reaction is yellow and positive reactions progress through several shades of green to a dark blue-green. The chemical reaction is the same glucose oxidase reaction, however.

11-13. The timing of the reactions is *critical* and *different* for each type of reagent strip. The multireagent, plastic strip (Labstix) must be read for glucose at 10 seconds after wetting. The continuous reagent strip (Tes-Tape) must be read first at 1 minute after wetting and 1 minute later (total 2 minutes) if the indication is 3+ or higher. The approximate concentration shown on the color chart should not be reported. The report should be 1+ or trace, and 2+, 3+, or 4+ as the color comparison indicated.

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[10] Synonyms for these generic names are:
(a) Tetracycline—Achromycin®, Panmycin®, Polycycline®, Tetracyn®.
(b) Oxytetracycline—Terramycin®.

11-14. You may have assumed that false reactions are not possible in this test since glucose oxidase is an enzyme specific for glucose. If you will look again at table 4, you will see that both false negative and false positive reactions have occurred. False negative reactions involving ascorbic acid, vitamin C, are most important because of its extensive use. As little as 250 mg. injected with an antibiotic is sufficient to inhibit the glucose oxidase reaction for glucose. Since the renal threshold for ascorbic acid is normally low, most of the vitamin is excreted in the urine. To complicate this difficulty even more, concentrations of vitamin C as low as 25 mg. % in urine react positively with the *Benedict test* or *reagent tablet*.

11-15. When vitamin C is present, you are faced with the dilemma of having *both* prime qualitative tests for glucosuria invalidated. Further, if you use a glucose oxidase reagent strip for initial screening, you will miss the reaction completely and not even be aware of it. The presence of ascorbic acid is not always so obvious as in its use as an antibiotic stabilizer. False negative reactions with the drugs dipyrone and meralluride, listed in table 4, are less important because of limited drug use. However, the fact that they have caused false negative tests for glucosuria prompts another question. What other substances now and in the future may cause false negative reactions with glucose oxidase?

11-16. The answer will be long in coming if the common practice of initial screening with glucose oxidase, alone, is continued. Benedict's, including the reagent test tablet, apparently has no known false negative reactions from interfering substances. In addition, some of the false glucose positive reactions may be clinically useful, i.e., alkaptonuria, salicylates, and pentosuria. The detection of galactosuria, though very rare, may even save an infant's life. It seems apparent, therefore, that a need for the Benedict sugar test in urinalysis still exists. From the record to date, the Benedict reaction on a urine specimen should not be entirely discounted on the basis of an opposite result with the glucose oxidase test.

12. Miscellaneous Urine Tests

12-1. In this concluding section we will discuss a variety of tests. Except for the fact that they are all performed on urine, there is no clearly defined relationship among these various tests. Included in this discussion are laboratory procedures for urobilinogen, porphyrins, homogentistic acid and melanin, bilirubin, ketones, phenylketonuria, and occult blood. In addition, we will present a brief resume of pregnancy testing, a subject to be expanded later in Career Development Course 90413.

12-2. **Urobilinogen.** The subject of bile pigments and related pathology was introduced in Chapter 2, Volume 2, of this course. You will recall that urobilinogen is produced in the intestine by the action of bacteria on bile pigments. Part of this compound is absorbed into the blood and excreted in small amounts in the urine. Increased concentrations are found in diseases associated with excessive red cell breakdown as well as in other conditions.

12-3. The urine sample must be fresh and protected from daylight since urobilinogen will be converted to urbilin upon standing. Urobilinogen (as well as other bile pigments) is detected by means of the Ehrlich reaction in which urobilinogen reacts with para-dimethyl-amino benzaldehyde in hydrochloric acid to form a red color. Bile pigments in the urine are first removed by absorption with calcium chloride or barium chloride. The procedure may be quantitated by the method of Wallace and Diamond in which Ehrlich's reagent is added to tubes containing serial dilutions of urine. The last tube showing a pink color is the endpoint.

12-4. Some investigators[11] consider the Wallace and Diamond test outdated, and prefer a modified Watson semiquantitative test. In the Watson method, Ehrlich's aldehyde reagent is reacted with urine after urobilin has been reduced back to urobilinogen with ascorbic acid. After formation of the aldehyde-urobilinogen complex, sodium acetate is added. The procedure is quantitated using the acid form of PSP (phenol red) dye. There appears to be no satisfactory quantitative procedure for urobilinogen that is uncomplicated and specific for urobilinogen. The addition of sodium acetate is advocated to enhance the color and, at least to some extent, inhibit color formation by indole and skatole derivatives.

12-5. A 1400- to 1600-hour specimen is usually used for this test. This is based on certain conclusions of Watson and coworkers that the output of urobilinogen is higher during this period. This is also a convenient time of the day to perform a test which must not be delayed. The urine must not be kept overnight. Perform the examination immediately, even though it is possible to reduce urobilin back to urobilinogen by the addition of ferrous hydroxide.

12-6. Urobilinogen is distinguished from porphobilinogen on the basis of chloroform solubility. Chloroform is added to the urine which has been reacted with Ehrlich's reagent. If the aqueous layer is red and the chloroform layer colorless, the test is positive for porphobilinogen. If the chloroform layer is red and the aqueous layer colorless, the test is positive for urobilinogen. If both layers are red, extract with chloroform until the chloroform layer is colorless. If the aqueous layer is still red, the test is positive for both porphobilinogen and urobilinogen.

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[11] Henry, R. J., *Clinical Chemistry*, Hoeber Med. Div., Harper & Row Publishers, 1964, p. 610.

12-7. **Prophyrins.** In our discussion thus far we have mentioned porphobilinogen. Actually, porphobilinogen is only one of a group of compounds classified as porphyrins or porphyrin precursors. Chemically, porphyrins are characterized by four pyrrole rings joined in a cyclic pattern by methene (CH) groups. Porphyrins form the basis of plant and animal respiratory pigments and involve very complex chemistry. In terms of clinical significance, porphyrins are usually divided into two major groups: (1) uroporphyrins and coproporphyrins which result from erythropoietic activity; and (2) porphobilinogen and 5-aminolevulinic acid (ALA) due to overproduction of these porphyrin precursors in the liver.

12-8. Normal urine contains small quantities of porphyrins (300 μg. per day for males and 200 μg. per day for females), principally coproporphyrins. There are various procedures for the quantitative determination of urine prophyrins and their precursors. The best methods are quite involved and are usually performed only at Air Force reference laboratories. Simple methods such as visual observation for color change of fluorescence under ultraviolet light are of limited usefulness.

12-9. **Homogentisic Acid and Melanin.** Porphyrins are by no means the only pigments detectable in urine. Porphyrinuria, or the excretion of porphyrins in urine, is a relative rare phenomenon. Although prophyrins impart an orange to red color to urine, abnormal coloration may be due to many other substances. An interesting and perhaps even more unusual clinical rarity is the brown to black color of *homogentisic acid* and the black color of *melanin (melanogen).* Homogentisic acid in the urine results from the disease *alkaptonuria* which is an error of phenylalanine and tyrosine (amino acid) metabolism. Homogentisic acid is usually detected by the darkening of urine upon becoming alkaline, the ferric chloride test, or other screening procedures and confirmed by paper chromatography. Melanin is a black pigment which is excreted in some cancer states (melanomas). The ferric chloride test is positive and confirmation of melanin is by means of chromatography. Darkening of urine also results from gentisic acid, indicans, and phenols.

12-10. **Urine Bilirubin.** There are several tests which can be used to detect bilirubin in urine. However, most Air Force laboratories use a bilirubin test kit which employs a diazo reagent tablet.[12] The test tablets contain stabilized p-nitrobenzene p-toluene sulfonate, sulfosalicylic acid, and sodium bicarbonate. The kit comes complete with a mat consisting of asbestos and cellulose fibers. When a urine sample containing bilirubin is added to the mat dropwise, the bilirubin remains on the surface of the mat. You should always use the mats provided rather than filter paper or some other substitute because of this absorbent quality.

12-11. A test tablet is then placed on the mat and 2 drops of water are allowed to flow over the surface of the tablet. You should not add more than 2 drops of water. The purpose of the sulfosalicylic acid and sodium bicarbonate is to produce effervescence which will enhance the solubility of the tablet. If the mat shows a blue or purple color within 30 seconds, the test is considered positive. A slight pink color within 30 seconds is negative. The test is reported simply as positive or negative. The Ictotest instruction sheet in the training aids package shows a positive and a negative reaction. The color of the tablet itself is not significant.

12-12. The test tablets just describe react with both free (indirect or unconjugated) and direct (conjugated) bilirubin. False positives and false negatives are rare. Urobilin will not react, nor will salicylates. The sensitivity range is between 0.05 and 0.1 mg. per 100 ml. of urine. This meets the diagnostic requirements in most pathological processes. Urine which contains dyes such as Pyridium or Serenium[13] may give a color reaction with bilirubin test tablets.

12-13. **Ketonuria.** The most commonly used tests for the detection of ketone bodies employ either *ketostix*® or *Acetest*® tablets.[14] Neither test detects β-hydroxybutyric acid, and *Ketostix*® strips are specific for acetoacetic acid. For practical purposes, there is no reason to distinguish among the ketone bodies. Hence, either of these two tests is adequate for clinical laboratory use.

12-14. In principle, the *Ketostix*® and *Acetest*® procedures are modifications of the Rothera test outlined in AFM 160–49, *Laboratory Procedures in Clinical Chemistry and Urinalysis.* That is, sodium ntiroprusside in a suitable medium will react with certain ketones to produce a purple color. In the *Acetest®*, 1 drop of urine is placed on a tablet and the color is noted after a specified time interval. The reaction is read and reported as a small, moderate, or large amount. The sticks follow a similar pattern and are reported in the same way. The same reagents are now available on Labstix[15] to measure ketones simultaneously with blood, glucose, protein, and pH. The colors appear as illustrated in the Labstix indicator.

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[12] The diazo test kit is a Federal stock listed item, FSN 6505-290-6031.

[13] Pyridium and Serenium—Registered trademarks.

[14] Ketostix and Acetest—Registered trademarks of the Ames Company, Inc., Elkhart, Indiana.

12-15. **Phenylketonuria (PKU).** The occurrence of phenylketonuria as an error of protein metabolism was mentioned in Chapter 3, Volume 2, of this course. Although the incidence of this disorder is only one in 20,000 births, the consequence of PKU are so serious that early screening is imperative. Reagent sticks are available for detecting phenylketones, principally phenylpyruvic acid. A reaction takes place between ferric ions and phenylpyruvic acid in a properly acidified medium. A false positive test frequently occurs, however. A pink color indicates the presence of salicylates. PAS also gives a color reaction.

12-16. More significantly, the use of a ferric reaction with urine is not adequate to detect phenylketonemia (phenylketones in the bloodstream), characteristic of this metabolic disorder at an early stage. Infants fail to show a positive urine test until several weeks after birth. Unless detected early, the infant suffers irreversible brain damage. Further, older infants with other symptoms of the disease do not always show a positive urine test even though the blood level of phenylketones is high.

12-17. Many states now require the Guthrie test as the acceptable screening test for phenylketones. The Guthrie test is based upon the principle that growth of *Bacillus subtilis* is inhibited by beta-2-thienylalanine; but in the presence of phenylalanine, inhibition is overcome and growth takes place proportional to the concentration of phenylalanine. Zones of inhibition are read around paper discs impregnated with blood from a heel puncture. Control discs containing measured quantities of L-phenylalanine are used for comparison. Although a legally required test in many states, the Guthrie test is a *screening* test, with many of the inherent difficulties of a bioassay method (e.g., temperature control, culture purity, standardization, etc.). Therefore, it is necessary to confirm a positive Guthrie test with a more specific quantitative analysis. The only presently reliable quantitative means is a fluorimetric method. Careful measurement of plasma phenylalanine is a necessary laboratory function in following the dietary treatment of this disorder. In any case, the use of reagent impregnated strips or the ferric chloride test is inadequate.

12-18. **Occult Blood.** The term "occult" means obscure or hidden. By occult blood we mean blood which cannot be grossly observed. Test strips are available through Air Force supply channels which detect both free hemoglobin and the hemoglobin that is released from blood by chemical action of the test strip. Hemoglobin catalyzes the oxidation of orthotolidine[16] by peroxide in the test strip, and a blue color results. Optimum pH for the reaction is provided by citrate buffers in the test strip. The reaction is observed at 1 minute and reported as small, moderate, or large amounts of blood according to the intensity of the blue color as illustrated in the Labstix indicator.

12-19. The test strips for blood should be stored in a cool area but not refrigerated. If the reagent test strip bottles contain a desiccant, it should be left in the bottle. It is important that strips be free of chemical contamination. Don't touch the test area of the strip or lay the strip on the workbench. In reading the color reaction, hold the strip close to the color chart under good lighting. Discolored test strips must not be used. The reagents described above are specific for hemoglobin and myoglobin, so false positives are rare. Ascorbic acid in the urine will inhibit the reaction and produce a false negative. Remember that antibiotics for injection contain large amounts of ascorbic acid which will produce urine levels high enough to interfere with this test.

12-20. **Pregnancy Tests.** Historically, a number of tests have been devised to determine pregnancy. The ancient Egyptians believed that urine from a pregnant woman would stimulate the growth of a seedling plant, and this was perhaps the first "laboratory test" for pregnancy. In more recent times at least a dozen scientifically invalid tests for pregnancy were performed on a wide scale before testing was placed on a sound physiological basis. These tests incorporated a wide variety of biologicals. Some of the reagents or biologicals used were colostrum, protozoa, bromine water, and placental extract. The principles underlying these procedures varied tremendously and none were considered reliable. Some diagnostic aids to pregnancy determination were actually dangerous from a medical standpoint. One of these, the contractile uterine test of Reel required the intravenous injection of posterior pituitary extract into the patient.

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[15] Labstix—Registered trademark of the Ames Company, Inc., Elkhart, Indiana

[16] Orthotolidine. Do not confuse orthotolidine with orthotoluidine, a similar compound.

12-21. It was Zondek who first demonstrated that a substance which induces ovulation in an immature mouse is produced by the placenta. The substance was later identified as a hormone, human chorionic gonadotropin (HCG). It was also discovered the HCG is present in the serum and urine of pregnant females after specialized hormone-producing cells of the placenta begin to function at the fifth or sixth day of pregnancy. The problem was to develop a test sufficiently accurate and sensitive to detect HCG in the blood or urine early in pregnancy.

12-22. At the time hormone production begins, the blood or urine level is not high enough to permit HCG detection. However, about the 21st day of pregnancy, the HCG level becomes measurable with most of the reliable tests. Since ovulation does not occur at the same point in the menstrual cycle with all patients, pregnancy does not occur at the same point in the cycle. Consequently, one has only an empirical rule in deciding how far along the suspected pregnancy might be in terms of days calculated from the last menstrual period. A currently accepted method such as the latex pregnancy test kit which is available as a standard item is sensitive from the 41*st* to the 109*th* day following the last menstrual period. After this, the level of HCG drops off below detectable limits.

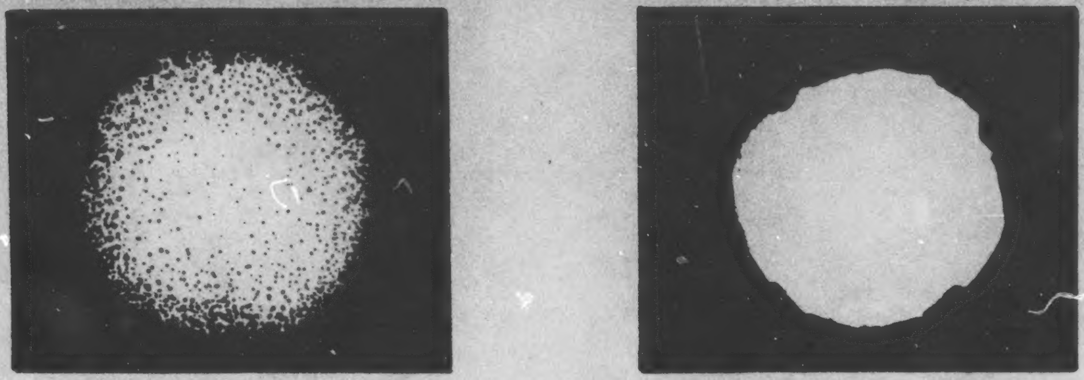
12-23. At the present time bioassay tests for pregnancy, i.e., tests utilizing experimental animals, must be considered largely of historical interest, but the technician should retain familiarity with the principles involved. Immunological techniques for pregnancy determinations are now receiving emphasis in clinical laboratories. The significant bioassay tests have been the Aschheim Zondek (A-Z), Friedman, and procedures using frogs and toads. In the Aschheim-Zondek test, urine containing gonadotropic hormones produces marked changes in the ovaries of female mice. Four days following the first injection, the mice are sacrificed. In a positive test the ovaries are enlarged, hyperemic, and show hemorrhagic spots and yellow protrusions (corpora lutea). If the test is negative the ovaries are small and white. Results of the test may be quantitatively reported in terms of mouse units of gonadotropin per 1,000 ml. of urine. The mouse unit was defined in Volume 3 of this course as the smallest quantity of estrus-producing hormone which will produce corpora hemorrhagica in the ovaries of immature female mice.

12-24. With urine from a male patient, the Aschheim-Zondek test is positive only in conditions associated with neoplastic tumors of the reproductive organs. Readings of less than 500 mouse units are considered of no significance, since in the common malignant tumors very large amounts of gonadotropic hormones are excreted in the urine. As a qualitative test for pregnancy, this procedure has many disadvantages. The method is lengthy and only specially trained technicians can effectively perform this test. The animals cannot be reused and often die due to injection trauma. Large estrogen doses may cause false negative tests, and menopausal urine may give a false positive test.

12-25. In the Friedman test, urine containing gonadotropic hormones produced marked changes in the ovaries of mature female rabbits. Two injections of 10 ml. each are given 24 hours apart. Forty-eight hours after the second injection, the rabbit is sacrificed and the ovaries are examined for the presence of hemorrhagic follicles which denote a positive test. Pale pink to white, unswollen ovaries are interpreted as a negative test. The rabbit or Friedman test is complicated and expensive, but when performed by experienced personnel it is very sensitive and at least 98 percent accurate. Menopausal urine has been reported to account for some of the false positive tests.

12-26. A number of tests utilizing amphibians have been developed. Two of these tests employ the male leopard frog, *Rana pipiens*, and female toad, *Xenopus laevis*. Male leopard frogs injected with urine or serum containing gonadotropic hormones void spermatozoa in their urine. The test requires 2 to 3 hours to read, and negative frogs should be injected with gonadotropin as a control measure. The test is reported to be 95 percent accurate, although some seasonal variation is experienced. In the spring, frogs have a low threshold and in the winter they have a higher threshold for the excretion of spermatozoa. Frogs are difficult to keep in a healthy state and frequently develop a bacterial infection termed "red leg." A number of drugs interfere with the frog test, including aspirin.

12-27. Female African toads *(Xenopus laevis)* will ovulate when injected with urine containing gonadotropic hormones. The toad is placed in a glass jar on wide-mesh, wire screen flooring suspended 1 inch from the bottom. Water is added to a depth of 3 inches. The toad sits on the screen and the gelatinous ova pass through the screen. These ova can be seen macroscopically and indicate a positive test. The test is reported negative if no ova are extruded after 24 hours. The African toad is quite difficult to procure and maintain, and a number of drugs interfere with this test.



NEGATIVE TEST (not Pregnant) **POSITIVE TEST (Pregnant)**

Figure 21. Plus or minus reaction to pregnancy test.

12-28. Serological tests for HCG are by far the most practical and widely used for the qualitative determination of human chorionic gonadotropin. Since HCG is antigenic, it has been possible to prepare an antiserum. Several immunologic methods have been developed to detect chorionic gonadotropin utilizing complement-fixation, agglutination-inhibition, and precipitin reactions. One of these, the slide immunologic test, is based on the principle of agglutination-inhibition. Latex particles coated with human chorionic gonadotropin serve as the antigen. One drop of the patient's urine is placed on a slide and mixed with a drop of chorionic gonadotropin antiserum. If the urine is from a nonpregnant individual (no gonadotropin present), the unchanged antiserum subsequently reacts with the coated latex particles to produce macroscopic agglutination. If the patient is pregnant the urine chorionic gonadotropin neutralizes the antiserum so that it is no longer free to react with the latex particles to bring about clumping.

12-29. You should observe certain precautions when you perform the pregnancy slide test. (Our remarks have special reference to the *Gravindex*®, a Federal stock listed item and a trade marked product of Ortho Diagnostics.) The slide should be rocked gently, not vigorously. Improper mixing of the specimen and the reagents may lead to erroneous results. The mixture must not be allowed to dry on the slide. The slide should be examined no longer than 2 minutes following addition of the antigen to the urine-antiserum mixture. Slides should not be placed on a warm surface such as the view box used in Rh testing. Positive urine may be used as a positive control. Samples of positive urine may be kept frozen in aliquots for convenient thawing and use.

12-30. Pregnancy test reagents must not be frozen, but maintained at refrigerator temperature when not in use. All reagents should be brought to room temperature before use. Any urine specimen, first in the morning or random, may be used. Specimens with a specific gravity of at least 1.015 are preferred for greater accuracy. Further, specimens should be tested within 12 hours of collection unless they are adequately preserved. Freezing is recommended for preservation. Do not attempt to test specimens which are grossly contaminated or which contain blood or abnormally high protein levels. A positive and negative reaction are shown in figure 21.

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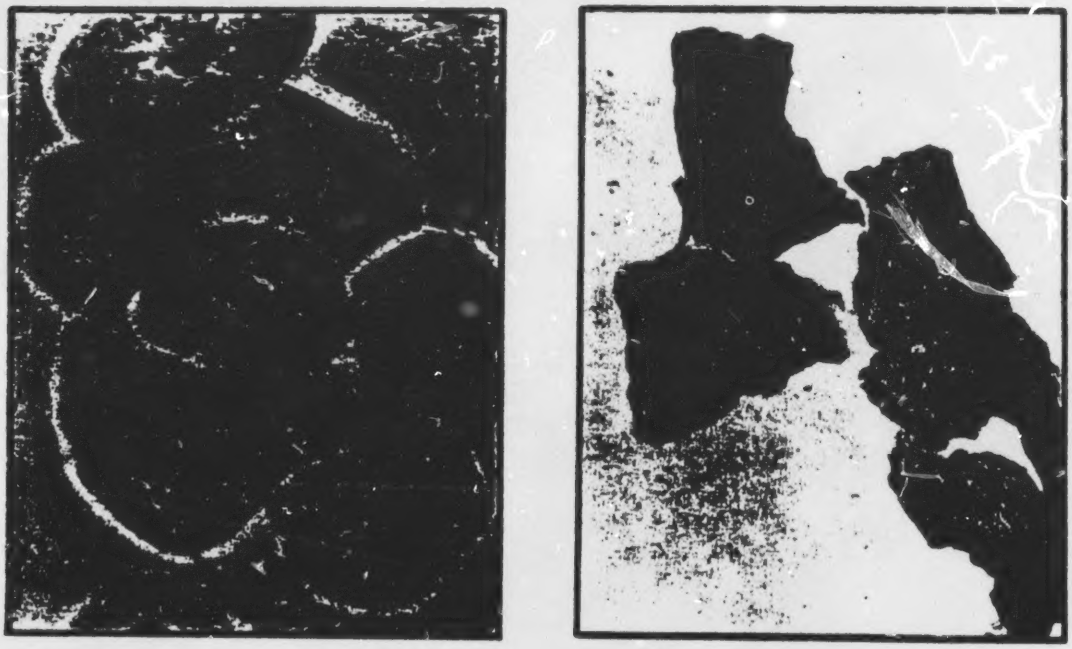

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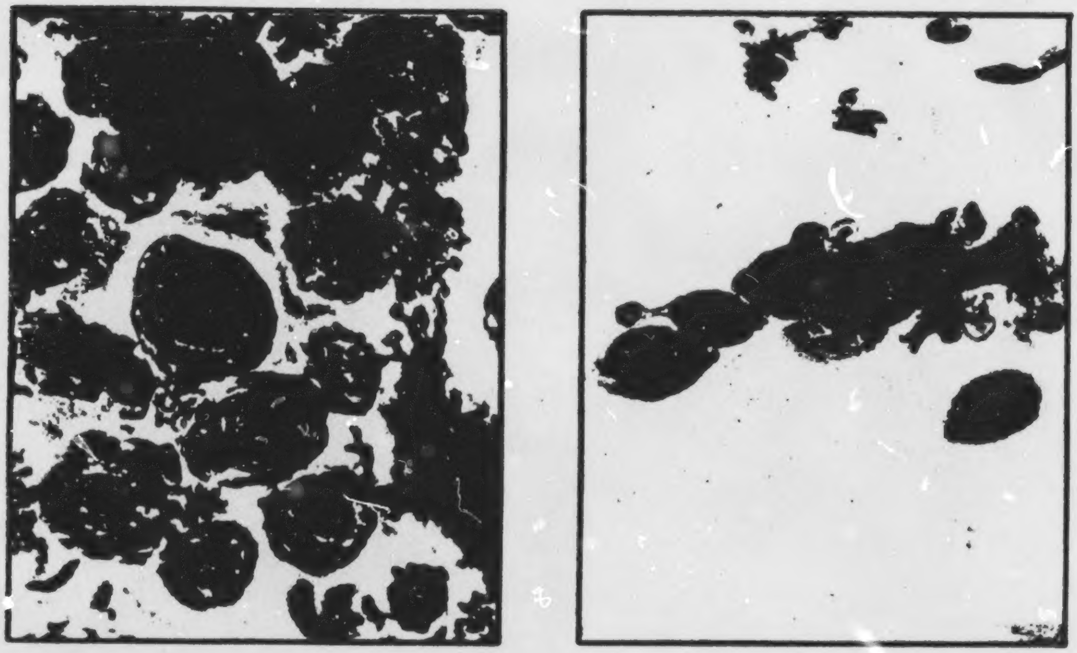

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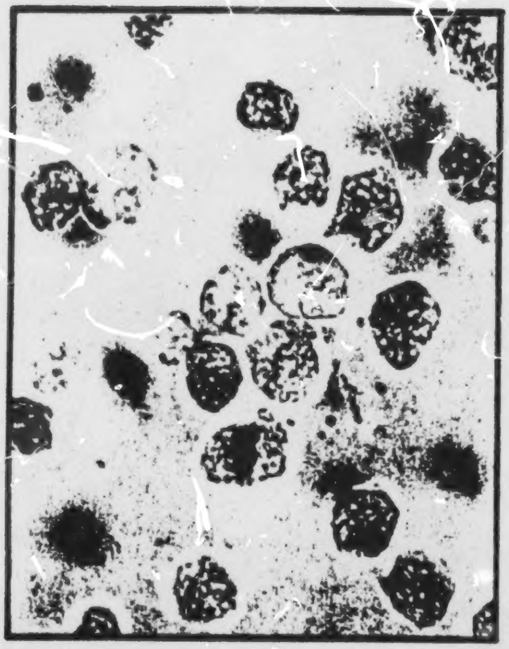

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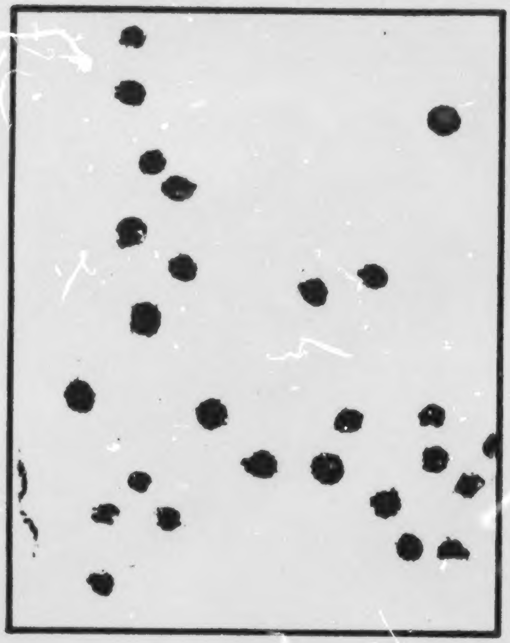

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OVAL FAT BODIES

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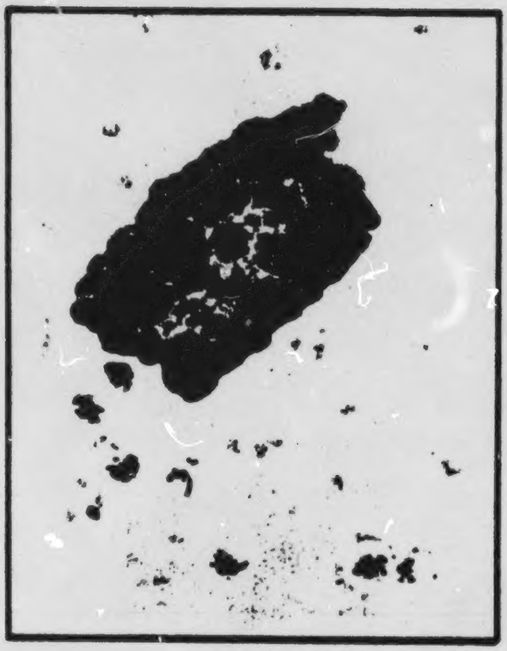

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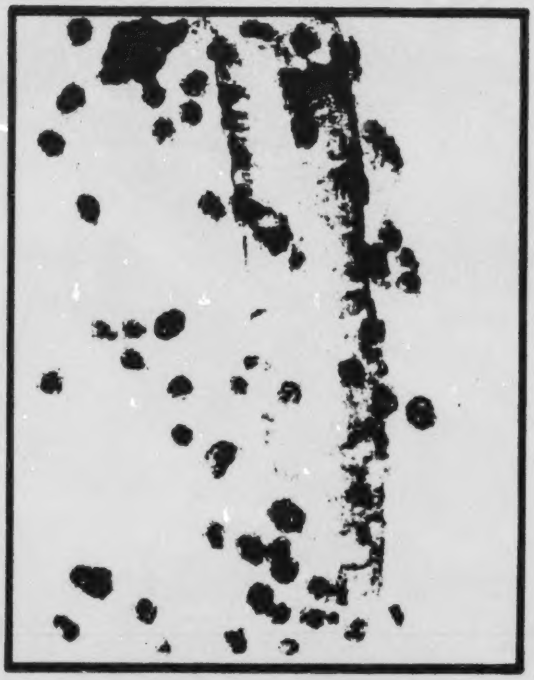

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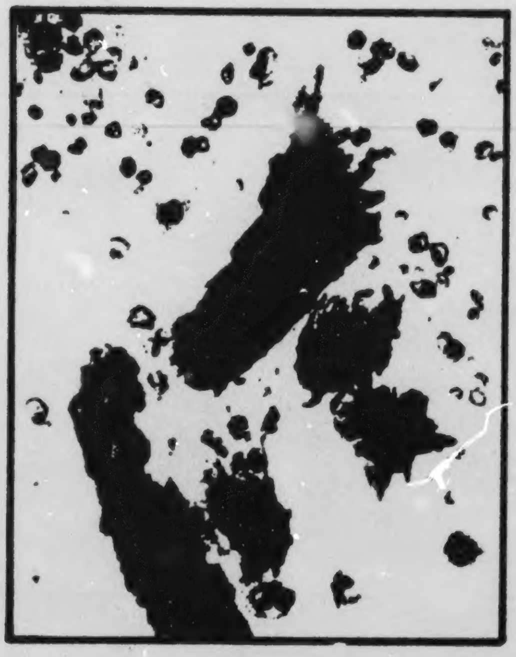

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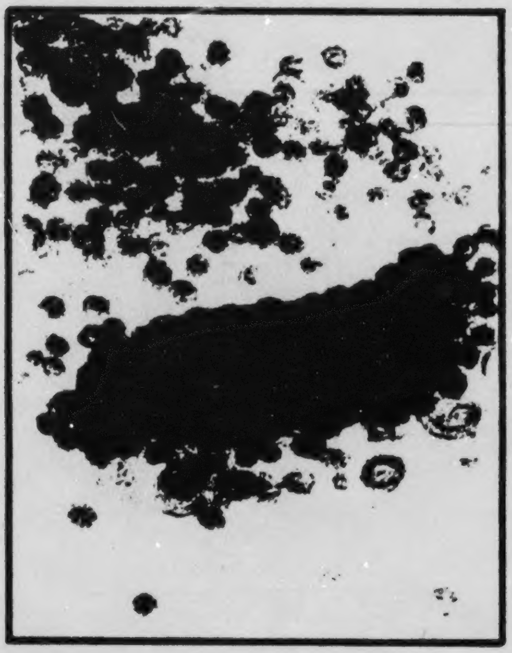

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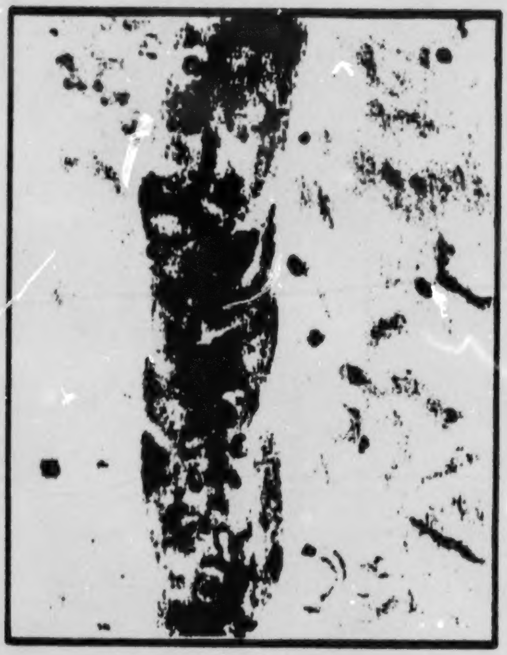

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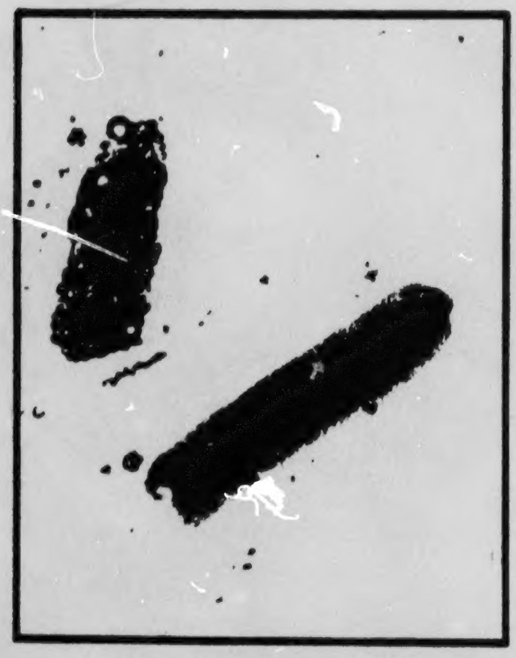

COARSE GRANULAR (UPPER LEFT)
WAXY CAST (LOWER RIGHT)

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ECI Form No. 17

STUDY REFERENCE GUIDE

1. *Use this Guide as a Study Aid.* It emphasizes all important study areas of this volume. Use the Guide for review before you take the closed-book Course Examination.

2. *Use the Guide for Follow-up after you complete the Course Examination.* The CE results will be sent to you on a postcard, which will indicate "Satisfactory" or "Unsatisfactory" completion. The card will list *Guide Numbers* relating to the items missed. Locate these numbers in the Guide and draw a line under the Guide Number, topic, and reference. Review these areas to insure your mastery of the course.

Guide Numbers 400 through 411

Guide Number	
400	Introduction to General Considerations; Review of Microscopy; pages 1-5
401	Collecting and Preserving Specimens; pages 5-8
402	Introduction to Renal Functions; Anatomy of the Kidney; pages 9-12
403	Physiology of the Kidney; pages 12-13
404	The Kidney and Disease; pages 13-14
405	Introduction to Physical Characteristics of Urine; Urine Volume, Color, and Appearance; pages 15-16
406	Reaction and Specific Gravity; pages 16-19
407	Introduction to Microscopic Examination; Organized Sediment; pages 20-26
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410	Urine Glucose; pages 35-39
411	Miscellaneous Urine Tests; pages 39-43

CHAPTER REVIEW EXERCISES

The following exercises are study aids. Write your answers in pencil in the space provided after each exercise. Immediately after completing each set of exercises, check your responses against the answers for that set. Do not submit your answers to ECI for grading.

CHAPTER 1

Objective: To understand the criteria required for proper use of the microscope, and to show a knowledge of laboratory activity with regard to the collection and preservation of urine specimens.

1. Describe how you would bring a microscope lamp into focus. (1-5,6)

2. *a.* How is the optimum position of the condenser selected?
   b. Is it acceptable to move the condenser to vary illumination when viewing a microscopic field?
   (1-7)

3. What other adjustment must be made by the Kohler method after the illuminating lamp and microscope condenser have been focused? (1-10)

4. *a.* What is the purpose of the iris diaphragm?
   b. For any given objective, how many "proper" settings are there for the iris diaphragm? Or would you say this depends upon the level of illumination desired?
   (1-10)

5. How would you control the intensity of illumination once your microscope and lamp system have been established for Kohler illumination? (1-15)

6. Essentially what does a three-glass urine microscopic examination tell the physician which may not be apparent from a one-specimen test? (2-6; Fig. 4)

7. *a.* List three urine preservatives commonly used.
   b. Which of the urine preservatives mentioned in the text gives a false positive test for albumin? For sugar?
   (2-10)

CHAPTER 2

Objective: To be able to describe structures of the kidney and related functions of clearing the blood and urine production.

1. What does a medullary ray consist of and where is it located within the kidney? (3-2)

2. List the four essential parts of a nephron unit. (3-4)

3. How does a change in blood pressure affect the rate of urine production? (4-3)

4. Where is most of the water reabsorbed in the kidney? (4-5)

5. Compare the normal pH and specific gravity of glomerular filtrate with the normal pH and specific gravity of urine. (4-4,6)

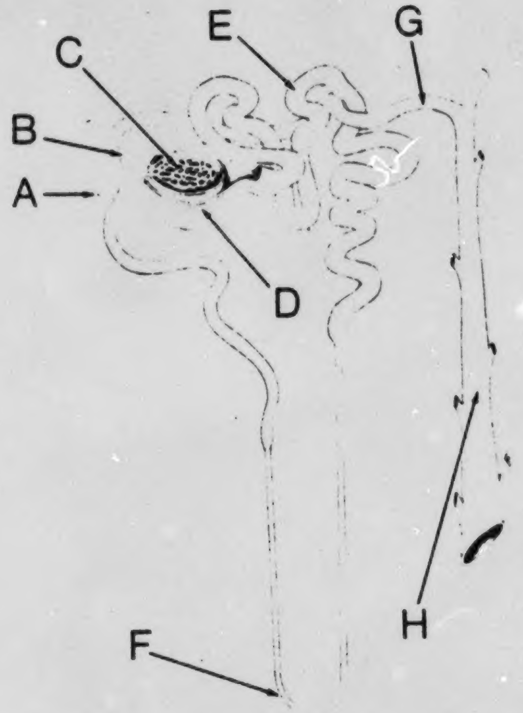
6. List four ways in which the kidneys are able to maintain electrolyte balance in the body. (4-7)

7. A physician tells you that one of his patients has "uremia." What does this mean? (5-2)

8. Why is it generally better to measure urea nitrogen than nonprotein nitrogen of the blood? (5-2)

9. What is the molecular weight of Bence-Jones protein and why is this an important point? (5-4)

10. Label the lettered parts in the following diagram. (Fig. 6)



A ______________________________

B ______________________________

C ______________________________

D ______________________________

E ______________________________

F ______________________________

G ______________________________

H ______________________________

CRE Figure 1.

CHAPTER 3

Objective: To be able to observe and report the physical characteristics of urine.

1. List three factors which affect the total urine volume. (6-1)

2. What is the average total daily urine volume voided by adults? (6-1)

3. Which urine specimen is the most valuable to a physician in a routine urinalysis? (6-2)

4. How may chromogens be removed from a urine specimen? (6-3)

5. When referring to the presence of hemoglobin in urine, what term is used? (6-4)

6. What assumptions of pathology can you make solely on the basis of urine color? (6-5)

7. What change occurs in the normal reaction of fresh urine upon standing at room temperature? (7-1)

8. Why might a physician intentionally alter the urine pH range of a patient? (7-4)

9. Why do we prefer not to use the older litmus paper technique in urine pH measurement? (7-6)

10. List three sources of error which may be encountered using pH paper strips. (7-7)

11. Why should the desiccant pack be left in a urine reagent test strip bottle? (7-8)

12. Define specific gravity. (7-9)

13. What temperature correction factor is used when you measure specific gravity with a urinometer? (7-10)

14. How would you correct the specific gravity reading for a threefold dilution of the specimen? (7-10,12)

15. What other instrument besides a hydrometer (urinometer) is used to measure the specific gravity of urine? (7-17)

CHAPTER 4

Objective: To be able to identify normal and abnormal urinary sediment.

1. What is meant by organized urinary sediment? (8-1)

2. *a.* How could you easily identify starch-containing granules which might appear in the urine?
   b. How are the yeast cells usually distinguished from other elements such as red blood cells?
   (8-2)

3. *a.* How many milliliters of urine should be centifuged.
   b. Is the amount important?
   c. Why or why not.
   (8-4)

4. The addition of dilute acetic acid is recommended to clear urine of amorphous phosphates. Is the amount added critical? Why or why not? (8-5)

5. How would you clear amorphous urates from urine? (8-5)

6. What is the current philosophy regarding the identification of epithelial cells according to site of origin? (8-7)

7. *a.* What are glitter cells, and why are they so-called?
   b. What are oval fat bodies, and what is their significance?
   (8-12)

8. List two ways to confirm the presence of observed red blood cells if the observation is in doubt? (8-14)

9. How would you distinguish granular casts with a hyalin matrix from granular casts derived from degenerated epithelial cells? (8-17,18; Table 1)

10. List and describe five types of inclusion casts. (8-18-21)

11. What is the origin of a waxy cast, and when is it most likely to be found? (8-22)

12. Of what significance is the size of a cast? (8-23)

13. What is the value as well as the limitation of classifying unorganized sediment on the basis of urine pH? (9-2)

14. Which of the following normally occur in alkaline urine?
    a. Triple phosphate.
    b. Ammonium biurate.
    c. Amorphous phosphates.
    d. Calcium oxalate.
    e. Uric acid.
    (9-3)

15. Describe examples of each of the following crystals:
    a. Triple phosphate. (9-4; Fig. 12)
    b. Ammonium biurate. (9-5; Fig. 13)
    c. Calcium oxalate. (9-8; Fig. 15)

16. List and describe three amino acid crystals sometimes found in urine. (9-9-13; Figs. 16-18)

CHAPTER 5

Objectives: To be able to list the qualitative screening tests used in urinalysis; to recognize the correct and false reactions which may occur; and to know the proper report to render.

1. What kind of chemical analysis is involved in screening urine specimens for the presence of a constituent? (Intro.-2)

2. What is semiquantitative analysis? (Intro.-2)

3. Where is protein exerted by the kidneys derived? (10-1)

4. What *nonserum* proteins are contributed to urine? (10-2)

5. List three causes of proteinuria which are not directly related to kidney disease. (10-3)

6. What are the two reasons for confirmatory testing of urine protein screening tests? (10-4)

7. What protein fraction is specifically measured by qualitative urine screening tests? (10-5)

8. What are the three most widely used urine protein screening tests? (10-7)

9. List three factors which influence the normal range of protein in a 24-hour urine specimen. (10-8)

10. Give the upper limits for normal urine protein in mg./100 ml. (10-9)

11. Why is the sensitivity of a urine protein screening test important? (10-10)

12. From the reactions you have studied, what can you say concerning the usefulness of the nitric acid ring test for protein? (10-11; Table 2)

13. What two properties of proteoses will eliminate them from interfering in tests for urine protein? (10-13)

14. Identify the steps in the heat and acetic acid procedure where the technician *must* observe the reaction. (10-14; Table 3)

15. If a very alkaline urine reacts positively on a reagent strip test for protein, what should you report? (10-15,16)

16. What are the prerequisites for a urine protein screening method? (10-17)

17. Why is a urine specimen centrifuged in the heat/acetic acid method for protein? (10-19)

18. Why is a buffer proposed for use in the heat/acetic acid test for urine protein? (10-20,21)

19. What is the principle of the reagent-impregnated strip for protein? (10-22)

20. Is the ideal (optimum) pH for protein reaction with the reagent strip acid or alkaline? (10-22)

21. How should you report a positive urine protein reaction from the reagent strip? (10-24)

22. What other portion of the reagent strip should be considered when you read the protein reaction of the strip? (10-25)

23. What is the best method for detecting diabetes mellitus? (11-1)

24. Give the causes for glucosuria, other than diabetes. (11-2)

25. Glucose, lactose, galactose, fructose, and pentoses are all in a group of sugars known as __________ . (11-3)

26. Benedict's test is a classic test for ______________ . (11-3,4)

27. What is the red chemical formed in a positive Benedict reaction with glucose? (11-5)

28. Which reagent is more chemically stable, the urine sugar test tablet or Benedict's reagent? (11-6)

29. Why must you observe the reaction when using a urine sugar test tablet? (11-7)

30. What is the primary criticism of copper reduction methods (Benedict's) for urine glucose screening? (11-9)

31. Name two reagents which may be used as known glucose specimens in the glucose oxidase urine screening test. (11-9,10)

32. Is the time lapse critical in reading a glucose oxidase strip reaction with urine? Why? (11-13)

33. What is the most important false urine glucose reaction in both glucose oxidase and copper reduction methods? Explain why it is. (11-14-16; Table 4)

34. Why must an analysis for urobilinogen be performed while the urine specimen is still fresh? (12-3)

35. What is the Ehrlich reaction? (12-3)

36. How would you distinguish between urobilinogen and porphobilinogen in terms of the Ehrlich reaction? (12-6)

37. Describe the reaction which takes place when a bilirubin test tablet is used with urine which is positive for bilirubin. (12-10)

38. Which ketone bodies are detected by *Ketostix*®? Which ketone bodies are detected by *Acetest*®? (12-13)

39. What is the principle of the *Ketostix*® and *Acetest*® procedures? (12-14)

40. *a.* What is phenylketonuria?
    b. What is the recommended technique for detecting PKU?
    c. Of what value are reagent sticks which use the ferric reaction with phenylpyruvic acid? (12-15-17)

41. What is the principle of the Guthrie test for PKU? (12-17)

42. State the principle of the hemoglobin test strip. (12-18)

43. What is the common feature of most modern, reliable pregnancy tests? (12-21)

44. Describe the principle of the agglutination-inhibition slide test for chorionic gonadotropin. (12-28; Fig. 21)

45. List some sources of error which apply to the *Gravindex*® slide test for pregnacy. (12-29,30)

ANSWERS FOR CHAPTER REVIEW EXERCISES

CHAPTER 1

1. Align the lamp so that it is directly in front of the microscope and is at a distance of approximately 8 to 12 inches from the microscope. By means of the knurled knob at the back of the lamp base, adjust the vertical position of the lamp so that the light falls on the center of the microscope mirror. The filaments of of the lamp bulb are sharply focused on the mirror with the filament focus control. Adjust the horizontal and vertical directions of the lamp so that the image of the filaments is centered on the mirror. An even more accurate method is to take a second flat mirror and position it adjacent to the microscope mirror so that one can see the underside of the condenser iris diaphragm. Close the condenser iris diaphragm so that its opening is but a pinpoint and the image of the lamp filaments is focused on the underside of the condenser iris diaphragm.

2. *a.* Check the microscope image of the slide object to be sure it is still in focus. With both the condenser iris diaphragm and the lamp iris diaphragm closed, move the microscope condenser up and down until a sharply defined image of the lamp iris diaphragm is obtained in the field of view. Once again the image of the object on the slide should be in sharp focus. Adjust the microscope mirror so that the image of the lamp iris diaphragm is centered in the middle of the field of vision. Open the iris diaphragm of the lamp until the field of vision seen through the microscope eyepieces is fully illuminated. The lamp iris diaphragm image should be centered and sharply defined in focus.
   b. No.

3. The condenser iris diaphragm must be properly adjusted.

4. *a.* The iris diaphragm is part of the optical system responsible for image resolution.
   b. There is only one correct setting for the iris diaphragm for each objective; this component is not intended to control the level of illumination.

5. The intensity of illumination is controlled with neutral density filters. Some lamps may be equipped with a rheostat which may also be used for this purpose.

6. A three-glass microscopic examination provides an index of where in the urinary tract certain formed elements are coming from.

7. *a.* Toluene, formalin, thymol.
   b. Thymol gives a false positive test for albumin. Formalin gives a false positive test for sugar if a reduction method is used.

CHAPTER 2

1. Medullary rays consist of branched collecting tubules and limbs of the loop of Henle. They are located in the renal cortex.

2. Glomerulus, proximal convoluted tubule, loop of Henle, and distal convoluted tubule.

3. In the absence of compensating factors, a rise in blood pressure increases the rate of glomerular filtration and hence increases urine production. A decrease in blood pressure has the opposite effect.

4. Proximal convoluted tubules.

5. Glomerular filtrate: pH 7.4; specific gravity 1.008 to 1.012. Urine: pH 4.6 to 8; specific gravity 1.015 to 1.025.

6. (1) Ketone bodies are oxidized to corresponding organic acids. (2) Ammonium ions replace sodium ions. (3) Bicarbonate ions are reabsorbed in the tubules. (4) Hydrogen ions are excreted by the tubules.

7. The term *uremia* indicates the presence of urinary constituents in the blood, usually urea nitrogen.

8. In measuring nonprotein nitrogen, we are measuring many miscellaneous substances including creatinine, amino acids, etc. It is more meaningful to be specific.

9. Bence-Jones protein has a molecular weight range of 25,000 to 90,000. This is an important point because molecules less than 68,000 a.m.u. readily pass thorugh the glomerulus.

10. A. Efferent arteriole.
    B. Afferent arteriole.
    C. Glomerulus.
    D. Bowman's capsule.
    E. Proximal convoluted tubule.
    F. Loop of Henle.
    G. Distal convoluted tubule.
    H. Collecting tubule.

CHAPTER 3

1. Diet, body size, fluid intake, fluid loss, diuretics.

2. 1,200 to 1,500 ml.

3. A freshly vioded, early morning specimen is usually the most valuable.

4. Chromogens may be removed by mixing the specimen with activated charcoal and filtering it.

5. Hemoglobinuria.

6. No assumptions can be made solely on the basis of urine color.

7. The reaction changes to alkaline because of the formation of ammonia from urea.

8. To control urinary calculi or to provide an effective pH range for antibiotic therapy.

9. A qualitative urine pH report (i.e. above or below pH 6.5) which is determined with litmus paper is of little value to the physician in clinical evaluation or treatment.

10. Acid or alkaline fumes, previously wet strips, and leaching reagent by soaking or excess wetting.

11. The desiccant is there to absorb moisture from the atmosphere which enters the bottle when it is open. Unless the reagent strips are kept dry they will not remain stable.

12. Specific gravity is the ratio of the weight or mass of a given volume of a substance to the weight of an equal volume of a standard, i.e., pure water.

13. 0.001 is added to or subtracted from the observed reading for each 3° C. higher or lower temperature, respectively, in relation to the calibration temperature.

14. The three digits after the decimal point would be multiplied by 3.

15. Refractometer.

CHAPTER 4

1. By *organized urinary sediment* we mean body cells and their derivatives which are found in urine. The definition could be expanded to include other cellular elements such as yeast, parasites, and bacteria; however, this is not the usual understanding.

2. *a.* Starch granules turn blue upon the addition of iodine.
   b. Yeast cells are usually identified by the presence of budding.

3. *a.* 10 ml.
   b. Yes.
   c. The amount will determine the quantitative aspect, i.e., how many constituents should normally be found.

4. The amount added should be minimal, although it is not really critical. If too much acetic acid is added, red blood cells and casts will be destroyed.

5. To clear amorphorus urates, add an equal part of warm water to the urine.

6. It is currently believed to be unwise for the laboratory technician to attempt identification of epithelial cells by site of origin.

7. *a.* Glitter cells are polymorphonuclear leukocytes. They are so-called because the engulfed bacteria glitter under light through the microscope.
   b. Oval fat bodies are intact epithelial cell borders which contain droplets of fat. They are clinically significant, and are especially characteristic of degenerative tubular disease.

8. Add acetic acid and if the cells disappear they were RBC's; second, the benzidine test for blood can be used to confirm the presence of RBC's.

9. Hyalin stains pink with the Sternheimer-Malbin stain.

10. *a.* Fine granular hyaline cast – more or less tightly packed fine granules in a hyalin matrix.
    b. Coarse granular hyaline cast – coarse granules in a hyalin matrix.
    c. Tabular epithelial cast – rounded epithelial cells in a hyalin matrix.
    d. Red cell – packed red cells in a hyalin matrix, and to be distinguished from a cast of cells without clear outlines (blood cast).
    e. Other – you might also suggest hyalin casts which contain crystals, bacteria, or fat.

11. A waxy cast is an epithelial cell cast which represents amyloid degeneration. It is found in urine of high specific gravity from patients with advanced nephritis and diseases characterized by amyloid deposition.

12. The size of a cast is some indication of the size of the lumen of the tubule in which the cast was formed. This is clinically significant because it is a clue to the inflammatory state of the tubule as well as an indication of the functioning capacity of the nephron units.

13. A classification of unorganized sediment based upon urine pH is helpful in crystal identification. Some crystals such as calcium oxalate may be present in either acid or alkaline urine; and this is a somewhat limiting factor in the usefulness of such a system.

14. *a*, *b*, and *c*.

15. *a*. Triple phosphate crystals may be three, four, or six sided, typically presenting the "coffin-lid" appearance.
    b. Ammonium biurate crystals show different forms including sheaves and dumbbells.
    c. Calcium oxalate crystals are eight sided with an "envelope" appearance. They may also appear as dumbbells or spheres.

16. *a*. Leucine -- yellow spheres presenting radial and concentric striations.
    b. Tyrosine -- fine needles arranged in sheaves.
    c. Cystine -- hexagonal plates with well-defined edges.

CHAPTER 5

1. Qualitiative analysis.

2. The chemical analysis which roughly estimates the quantity of a chemical constituent.

3. From plasma proteins.

4. Nonserum proteins in urine are mucoproteins.

5. *a*. Extradinary physical exercise.
   b. Ingestion of high protein meal.
   c. Postural aberration.
   d. Febrile conditions.
   e. Bleeding in urogenital tract.

6. *a*. The clinical importance of the result.
   b. The limitations of any single method.

7. No qualitiative urine protein screening test measures a specific protein fraction.

8. *a*. An indicator with buffer.
   b. Heat and acetic acid.
   c. Sulfosalicylic acid.

9. *a*. 24-hour urine volume.
   b. Diet.
   c. Urinalysis method.
   d. Normal physiological fluctuations.

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10. 7 to 10 mg./100 ml. of urine.

11. If the screening test is so sensitive that it detects less than the higher limits or normal (7 to 10 mg. percent), positive results will be obtained on normal amounts of urine protein. This would render the test useless for screening urine specimens.

12. The nitric acid ring test for protein reacts falsely with many substances other than protein. It is a nonspecific reaction and practically worthless as a screening test.

13. Proteoses will dissolve in picric acid or heated urine; however, protein precipitates in both cases and is thus specifically identified.

14. *a.* Initial observation of uncentrifuged urine.
    b. After centrifugation.
    c. After initial boiling.
    d. After acidification.
    e. After reboiling.

15. A highly buffered alkaline urine specimen will give a false positive protein reaction. You should not report this reaction unless it is confirmed by a positive reaction with heat and buffered acetic acid.

16. *a.* Sensitive enough to detect all abnormally high quantities of urine protein.
    b. Not so sensitive that it reacts with normal levels of urine protein.
    c. Few, if any, false reactions.
    d. Stable reagents.
    e. Efficient procedure.

17. Centrifugation removes most of the crystals and cellular components of urine so that they will not interfere with the reaction.

18. A buffer in the heat/acetic acid method will maintain the optimal pH of the reaction for protein precipitation between pH 4 and 5.

19. Certain pH indicators will turn different colors at the same pH in the presence of protein.

20. Acid, pH 3.

21. Trace, 1+, 2+, 3+, or 4+.

22. The pH portion should be considered because of the possibility of a false positive protein in an alkaline urine.

23. Screening urine specimens for glucosuria.

24. *a.* Kidney disease.
    b. Pancreatic disease.
    c. Endocrine disorders.
    d. Damage to central nervous system.
    e. Stress.
    f. Pregnancy.
    g. Anesthesia.

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25. Reducing sugars.

26. Reducing sugars.

27. Cuprous oxide (CU_2O).

28. Benedict's reagent.

29. In case there is a pass through reaction indicating reducing substances greater than 2 percent. This will not be obvious after the reaction has occurred.

30. There are many false glucose reactions.

31. *a.* An equilibrated glucose solution from shelf reagent.
    b. Coca Cola.

32. Yes. It must be read at the times indicated in the directions for each method.

33. The most serious false reaction in urine glucose screening with glucose oxidase and copper reduction methods is from vitamin C (ascorbic acid). Concentrations encountered from the therapeutic levels will cause false negative glucose oxidase reactions and false positive copper reduction tests for glucose. If glucose oxidase is used as the initial screening test, positive glucosuria will be missed entirely.

34. Urobilinogen will disappear from urine, as it is readily oxidized to urobilin.

35. Urobilinogen and other bile pigments react with paradimethyl-amino benzaldehyde in hydrochloric acid to form a red color.

36. Porphobilinogen can be extracted with chloroform; the red urobilinogen complex is not soluble in chloroform.

37. Bilirubin reacts with p-nitrobenzene p-toluene sulfonate and imparts a purple color to a mat of asbestos and cellulose fibers upon which the tablet is placed. The tablet also contains a weak acid and a base to enhance solubility of the reactants.

38. *Ketostix®* detects only acetoacetic acid. *Acetest®* detects both acetone and acetoacetic acid.

39. Sodium nitroprusside reacts with certain ketones in a suitable medium to produce a purple color.

40. *a.* Phenylketonuria is an error of protein metabolism which results in the accumulation of phenylketones in the blood and consequent mental retardation.
    b. PKU is detected by means of the Guthrie test or other suitable means.
    c. The reagent sticks are of very little value and should not be used except as a rough screening procedure in addition to more reliable tests.

41. Phenylalanine prevents the inhibition of a culture of *Bacillus subtillis* by beta-2-thienylalanine.

42. The test strips for hemoglobin are impregnated with orthotolidine which turns blue when oxidized by peroxide in the presence of hemoglobin.

43. Most, if not all, modern reliable pregnancy tests measure chorionic gonadotropin.

44. The patient's urine is mixed with anti-HCG. If the urine contains no HCG, there is no reaction. If the urine does contain HCG, the anti-HCG is neutralized. The anti-HCG reagent is then reacted with latex particles coated with HCG. Lack of noticeable agglutination signals previous neutralization of the anti-HCG and is considered a positive test.

45. Improper mixing of specimen and reagents; drying on the slide; failure to use control; not conducting the test at room temperature; allowing the specimen to deteriorate; testing contaminated specimens; errors in reading the test.

STOP–

1. **MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.**
2. **USE NUMBER 1 PENCIL.**

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VOLUME REVIEW EXERCISE

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that numerical sequence on answer sheet alternates across from column to column.
3. Use only medium sharp #1 black lead pencil for marking answer sheet.
4. Circle the correct answer in this test booklet. After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than with a #1 black lead pencil.

NOTE: TEXT PAGE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Text Page Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Text Pages* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual VRE items you missed.* Go to the VRE booklet and locate the *Text Page Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (001-002) Prior to establishing Kohler illumination with the binocular microscope, you should

   a. align the condenser.
   b. adjust the diaphragm.
   c. focus the lamp filaments.
   d. adjust for refractive differences of your two eyes.

2. (004) To produce the desired reduction in illumination of the back lens, you should adjust the

   a. 10X objective.
   b. condenser iris diaphragm.
   c. neutral density filters.
   d. color correction filter.

3. (004) The intensity of microscopic illumination should be controlled

   a. with adjustment of the lamp iris.
   b. by closing the condenser iris.
   c. with neutral density filters.
   d. by opening the condenser iris.

4. (005) Hyaline casts dissolve most readily in urine of

   a. low specific gravity and high pH.
   b. low specific gravity and low pH.
   c. high specific gravity and high pH.
   d. high specific gravity and low pH.

5. (007) One of the disadvantages in using formalin as a specimen preservative is that it

   a. contains carbon atoms.
   b. volatilizes.
   c. inhibits the test for indican.
   d. extracts steroids.

6. (007) Which of the following gives a false positive test for albumin?

   a. Toluene.
   b. Thymol.
   c. Formalin.
   d. Xylene.

7. (009) The medullary rays of the kidney are located principally within the

   a. medulla.
   b. capsule.
   c. hylum.
   d. cortex.

8. (010) Structures directly related to, but not a part of, the kidney include

   a. ureters.
   b. collecting tubules.
   c. glomeruli.
   d. *ducts of Bellini.*

9. (010-011) Blood is supplied to each nephron unit of the kidney through

   a. the inferior vena cava.
   b. an efferent arteriole.
   c. an afferent arteriole.
   d. the renal vein.

10. (012) The normal average specific gravity of glomerular filtrate is

    a. 1.006.
    b. 1.010.
    c. 1.015.
    d. 1.025.

11. (012-013) Most of the water in glomerular filtrate is absorbed in the

a. proximal convoluted tubules.
b. glomerulus.
c. distal convoluted tubules.
d. loop of Henle.

12. (013) The normal volume of urine excreted by an adult in liters per 24 hours averages

a. 0.5 to 1.0.
b. 1.0 to 1.5.
c. 1.5 to 2.0.
d. 2.0 to 2.5.

13 (013) A term which indicates the presence of urinary constituents in the blood is

a. uremia.
b. acidosis.
c. uroerythria.
d. uriticaria.

14. (014) Renal amyloidosis is an aspect of which tubular disorder?

a. Mercury poisoning.
b. Tuberculosis.
c. Multiple myeloma.
d. Gout.

15. (014) Which vascular disease primarily involves the capillaries?

a. Nephrosclerosis.
b. Glomerulonephritis.
c. Glomerulosclerosis.
d. Thrombosis.

16. (015) The normal daily output of urine for adults should not exceed

a. 3,000 ml.
b. 2,000 ml.
c. 1,500 ml.
d. 1,000 ml.

17. (015) Variation among individuals in 24-hour urinary volume does *not* depend upon

a. fluid intake.
b. body size.
c. diet.
d. renal threshold.

18. (015) The most valuable urine specimen to a physician is usually

a. a late-evening specimen.
b. a mid-afternoon specimen.
c. a first-morning specimen.
d. one taken at random.

19. (016) When the urine has red color in it,

a. you may assume the presence of pathology.
b. you should attribute the variation to the patient's diet.
c. you may assume that the blood is normal.
d. you should be alerted to the possibility of pathology.

20. (016) An aged urine specimen becomes alkaline because of the formation of

a. ammonia.
b. *Pseudomonas.*
c. urea.
d. *Proteus.*

21. (017) Leaching of reagent from a paper strip occurs when the strip is

a. drained.
b. soaked.
c. dipped.
d. touched.

22. (017) To maintain the stability of reagent-impregnated strips, the screw-capped bottle should

a. remain open.
b. be refrigerated.
c. contain desiccant.
d. be stored in a dark room.

23. (017) In using a urinometer to measure specific gravity, the correction factor for each 3° C. higher or lower than calibration temperature is

a. ±1.001.
b. ±0.100.
c. ±0.010.
d. ±0.001.

24. (020) An object in urine which appears to be budding is probably a

a. yeast cell.
b. spermatozoan.
c. white blood cell.
d. dividing erythrocyte.

25. (021) The amount of urine in milliliters which is normally centrifuged for a urine microscopic examination is

a. 5.
b. 8.
c. 10.
d. 15.

26. (021) The addition of dilute acetic acid will clear the urine of

a. uric acid.
b. amorphous carbonates.
c. amorphous urates.
d. leukocytes.

27. (022) Glitter cells in urinary sediment

a. have no diagnostic value.
b. stain orange with Sudan III.
c. are characteristic of degenerative tubular disease.
d. contain many bacteria.

28. (023) In a hypertonic medium, red blood cells will

a. hemolyze.
b. remain intact.
c. produce *ghost* forms.
d. crenate.

29. (023-024) Another term for *transudation* in describing a cast is

a. epithelial.
b. waxy.
c. hyaline.
d. transitional.

30. (024) With the Sternheimer-Malbin stain, hyaline material stains

a. blue.
b. green.
c. red.
d. pink.

31. (024) With the Sternheimer-Malbin stain, red cells in a red cell inclusion cast appear

a. pale lavender.
b. dark blue.
c. pale pink.
d. orange.

32. (025) With the Sternheimer-Malbin stain, fat will produce

a. a pink stain.
b. a red stain.
c. a blue stain.
d. no stain.

33. (025) Broad casts in urine

a. result from urinary stasis.
b. indicate a swollen tubular epithelium.
c. occur primarily in urine from children.
d. result from all of the above.

34. (027) "Highly refractile prisms varying in size and resembling coffin lids" describes

a. triple phosphate crystals.
b. ammonium oxalate.
c. ammonium biruate.
d. uric acid.

35. (027-028) Amorphous urates may be differentiated from amorphous phosphates on the following criterion,

a. amorphous urates are soluble in acetic acid.
b. amorphous urates are soluble in alkali.
c. amorphous phosphates dissolve more readily upon warming.
d. amorphous phosphates are soluble in alkali.

36. (028) Leucine and tryosine crystals are

a. commonly found in the urine.
b. both soluble in dilute hydrochloric acid.
c. both soluble in boiling acetic acid.
d. formed as the result of serious liver damage.

37. (028) The Morner reagent is used to test for

a. tyrosine.
b. cystine.
c. leucine.
d. sulfa derivatives.

38. (028-029) A solution will give a red color with naphthoquinone-4-sodium sulfonate sulfite reagent (Sullivan test) if it contains

a. leucine.
b. isoleucine.
c. cystine.
d. cholesterol.

39. (029) When cystals of the sulfonamides are found in urine, they usually indicate

a. faulty metabolism.
b. toxicity.
c. alkaline urine.
d. sulfa therapy.

40. (030) The relatively small protein molecule, albumin, has a molecular weight of approximately

a. 70,000.
b. 7,000.
c. 700.
d. 70.

41. (031) Accounting for normal fluctuations in urine volume, the upper limit of "normal" for urinary protein in mg/100 ml. of urine is

a. 1 to 15.
b. 7 to 10.
c. 7 to 70.
d. 70 to 100.

42. (035) In the heat and acetic acid screening test for urine protein, the acetic acid

a. reacts with carbonates.
b. dissolves urates.
c. forms an acid reagent buffered at pH 4.
d. prevents negative reactions.

43. (035-036) Which of the following will *not* reduce Benedict's reagent?

a. Xylose.
b. Sucrose.
c. Lactose.
d. Glucose.

44. (037-038) A "pass through" reaction with the urine sugar test tablet indicates that reducing substances are

a. negative.
b. 1 percent or less.
c. 2 percent.
d. more than 2 percent.

45. (038) Ascorbic acid, which interferes with urine glucose tests, may result from

a. endocrine disorders.
b. iron therapy.
c. abnormal amounts of protein.
d. a diseased pancreas.

46. (038) How long does it take to establish a chemical equilibrium between the alpha and beta forms of *D*-glucose in water?

a. 5 minutes.
b. 15 minutes.
c. 30 minutes.
d. 1 hour.

47. (039) What substance below causes a false negative glucose oxidase reaction and false positive Benedict's reaction?

a. Lactose.
b. Coco Cola.
c. Vitamin C.
d. Antibiotics.

48. (039) The urine sample must be fresh when making the urobilinogen test because if it stands too long, it is converted to

a. stercobilinogen.
b. mesobilinogen.
c. bilirubin.
d. urobilin.

49. (040) Which of the following is *not* one of the porphyrin group of compounds?

a. Porphobilinogen.
b. Urobilinogen.
c. Coproporphyrin.
d. 5-aminolevulinic acid.

50. (040) Which of the following contains a black pigment excreted in some cancer states?

a. Gentisic acid.
b. Indican.
c. Melanin.
d. Phenol.

51. (040-041) The reagent used in the Acetest® (or modified Rothera test) is

a. sodium azide.
b. sodium nitroprusside.
c. para-benzaldehyde.
d. diazo reagent.

52. (041) As a test for PKU, test sticks employing the ferric ion reaction with phenylpyruvic acid are best described as

a. adequate for screening.
b. semiquantitative.
c. inadequate.
d. better than the Guthrie test.

53. (042) A bioassay test for gonadotropin which uses female mice is the

a. Aschheim-Zondek.
b. Friedman.
c. *Xenopus laevis.*
d. *Rana pipiens.*

54. (043) Which of the following terms best describes the slide test for pregnancy involving latex particles coated with human chorionic gonadotropin?

a. Agglutination.
b. Agglutination-inhibition.
c. Precipitation.
d. Antolytic.

55. (043) Which of the following is *not* a source of error in performing the *Gravindex®* slide test for pregnancy?

a. Using reagents which are at refrigerator temperature.
b. Vigorously mixing the specimen.
c. Viewing the slide on an Rh-type viewbox.
d. Discarding the reaction mixture after 2 minutes.

56. (043) Urine specimens for pregnancy testing that must be preserved are best preserved by

a. freezing.
b. boric acid.
c. formalin.
d. refrigeration.

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SUPPLEMENTARY MATERIAL

CDC 90411

MEDICAL LABORATORY TECHNICIAN - CLINICAL CHEMISTRY AND URINALYSIS

Volumes 1 thru 4

HANDBOOK OF REFERENCE MATERIAL

Extension Course Institute
Air University

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For purposes of this career development course, you will not be responsible for information included in this pamphlet *unless* it is repeated, expanded, or otherwise developed in the text of the various chapters. The supplementary information is intended as reference material which will be helpful in this course and in your daily work. It is divided into three separate appendixes. Appendix A provides information on the elements, and also explains how to obtain valuable data from a periodic chart. It relates specifically to Chapters 1 and 2 of Volume 1 and recurrently to various aspects of the clinical chemistry laboratory. Appendix B serves as a handy reference of clinical chemistry values with pertinent special instructions. The list which is presented will be of value on many occasions in your career. However, remember that clinical chemistry is a rapidly progressing field; and it is, therefore, necessary to constantly revise and update data of this type. Also, "normal values" vary with current concepts and sometimes with the test itself. Finally, Appendix C is a brief but quite detailed review of mathematics, including that time-saving device, the slide rule. The summary of general formulae presented here is just that -- a summary. Detailed discussion and a similar table may be found in AFM 160-49, *Laboratory Procedures in Clinical Chemistry and Urinalysis*. As pointed out in the text, chemistry is best expressed mathematically. Time devoted to developing facility in mathematics is well spent. A few sheets of graph paper and a standard deviation worksheet are included which are to be used in your solution to some of the problems.

APPENDIXES

APPENDIX A. THE ELEMENTS
    Section 1. Table of Elements Common to the Clinical Laboratory
    Section 2. Periodic Chart and Electron Configuration Table

APPENDIX B. TABLE OF CLINICAL CHEMISTRY VALUES
    Section 1. Analyses Performed on Serum
    Section 2. Analyses Performed on Urine
    Section 3. Collection and Shipment of Urine Specimens
    Section 4. Analyses Performed on Spinal Fluid
    Section 5. Bibliography to Appendix B

APPENDIX C. MATHEMATICS
    Section 1. Equations
    Section 2. Exponents
    Section 3. Logarithms
    Section 4. Review of the Slide Rule
    Section 5. Graphs
    Section 6. Summary of General Formulae and Concentrated Reagent Table
    Section 7. Graph Paper
    Section 8. Standard Deviation Worksheet

APPENDIX A. THE ELEMENTS

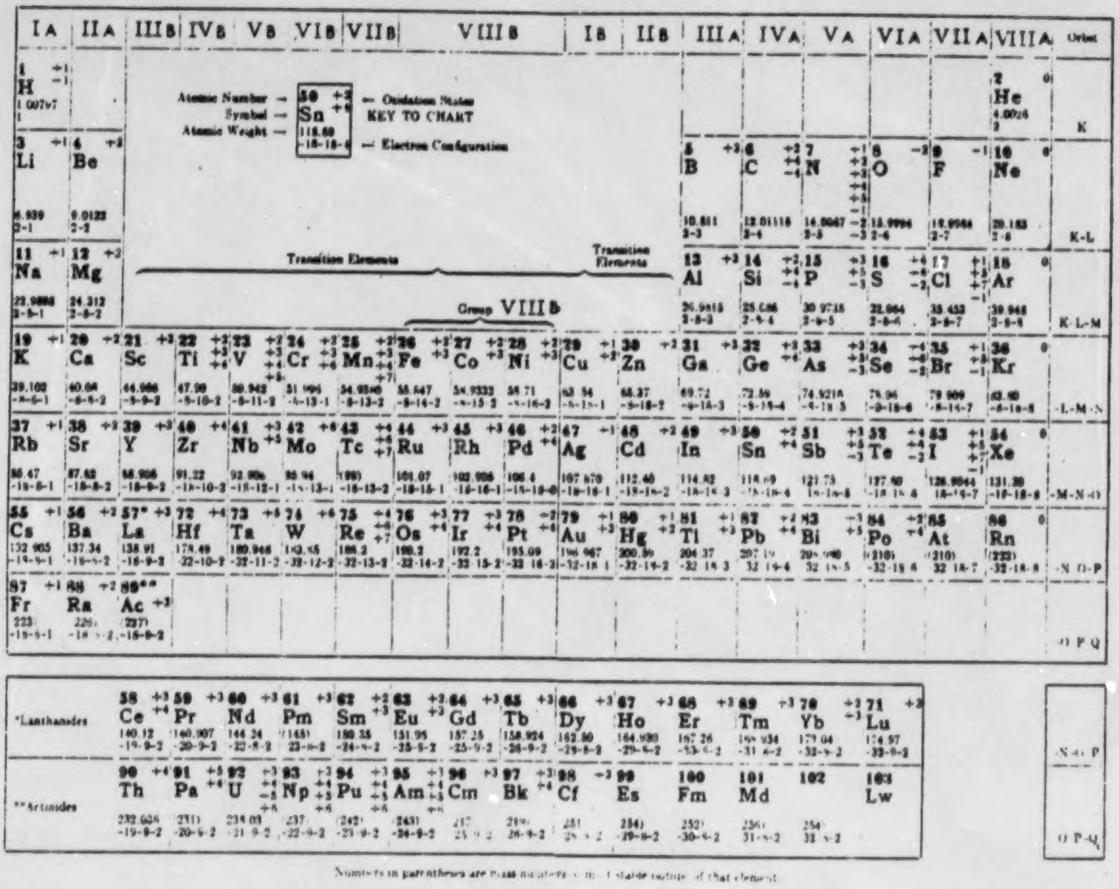
Section 1. *Table of Elements Common to the Clinical Laboratory*

This table provides a rapid means of referring to the atomic weight or atomic number of elements frequently encountered in the clinical laboratory.

NAME	*SYMBOL*	*ATOMIC NO.*	*ATOMIC WT.*
Aluminum	Al	13	26.98
Antimony	Sb	51	121.76
Arsenic	As	33	74.91
Barium	Ba	56	137.36
Bismuth	Bi	83	209.00
Bromine	Br	35	79.92
Calcium	Ca	20	40.08
Carbon	C	6	12.01
Chlorine	Cl	17	35.46
Chromium	Cr	24	52.01
Cobalt	Co	27	58.94
Copper	Cu	29	63.54
Fluorine	F	9	19.00
Gold	Au	79	197.00
Hydrogen	H	1	1.008
Iodine	I	53	126.91
Iron	Fe	26	55.85
Lead	Pb	82	207.21
Lithium	Li	3	6.94
Magnesium	Mg	12	24.32
Manganese	Mn	25	54.94
Mercury	Hg	80	200.61
Molybdenum	Mo	42	95.95
Nickel	Ni	28	58.71
Nitrogen	N	7	14.01
Osmium	Os	76	190.20
Oxygen	O	8	16.00
Palladium	Pd	46	106.4
Phosphorus	P	15	30.98
Potassium	K	19	39.10
Selenium	Se	34	78.96
Silicon	Si	14	28.09
Silver	Ag	47	107.88
Sodium	Na	11	22.99
Sulfur	S	16	32.07
Tin	Sn	50	118.70
Tungsten	W	74	183.86
Zinc	Zn	30	65.38

Section 2. *Periodic Chart**

The periodic law states that chemical properties of elements are periodic functions of their atomic numbers. That is, if elements are arranged according to atomic number, they can be grouped in vertical columns in such a way that each element will have properties similar to the one directly above or below it. Following the Periodic Chart is a table showing the electron configuration of the elements of the first three periods.



KEY TO CHART
Atomic Number → 50 +2 +4 ← Oxidation States
Symbol → Sn
Atomic Weight → 118.69
-18-18-4 ← Electron Configuration

Transition Elements
Group VIIIB

IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIIIB			IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	Orbit
1 +1 −1 H 1.00797 1																	2 0 He 4.0026 2	K
3 +1 Li 6.939 2-1	4 +2 Be 9.0122 2-2											5 +3 B 10.811 2-3	6 +2 +4 −4 C 12.01115 2-4	7 +1 +2 +3 +4 +5 −1 −2 −3 N 14.0067 2-5	8 −2 O 15.9994 2-6	9 −1 F 18.9984 2-7	10 0 Ne 20.183 2-8	K-L
11 +1 Na 22.9898 2-8-1	12 +2 Mg 24.312 2-8-2											13 +3 Al 26.9815 2-8-3	14 +2 +4 −4 Si 28.086 2-8-4	15 +3 +5 −3 P 30.9738 2-8-5	16 +4 +6 −2 S 32.064 2-8-6	17 +1 +5 +7 −1 Cl 35.453 2-8-7	18 0 Ar 39.948 2-8-8	K-L-M
19 +1 K 39.102 -8-8-1	20 +2 Ca 40.08 -8-8-2	21 +3 Sc 44.956 -8-9-2	22 +2 +3 +4 Ti 47.90 -8-10-2	23 +2 +3 +4 +5 V 50.942 -8-11-2	24 +2 +3 +6 Cr 51.996 -8-13-1	25 +2 +3 +4 +7 Mn 54.9380 -8-13-2	26 +2 +3 Fe 55.847 -8-14-2	27 +2 +3 Co 58.9332 -8-15-2	28 +2 +3 Ni 58.71 -8-16-2	29 +1 +2 Cu 63.54 -8-18-1	30 +2 Zn 65.37 -8-18-2	31 +3 Ga 69.72 -8-18-3	32 +2 +4 Ge 72.59 -8-18-4	33 +3 +5 −3 As 74.9216 -8-18-5	34 +4 +6 −2 Se 78.96 -8-18-6	35 +1 +5 −1 Br 79.909 -8-18-7	36 0 Kr 83.80 -8-18-8	-L-M-N
37 +1 Rb 85.47 -18-8-1	38 +2 Sr 87.62 -18-8-2	39 +3 Y 88.905 -18-9-2	40 +4 Zr 91.22 -18-10-2	41 +3 +5 Nb 92.906 -18-12-1	42 +6 Mo 95.94 -18-13-1	43 +4 +6 +7 Tc (99) -18-13-2	44 +3 Ru 101.07 -18-15-1	45 +3 Rh 102.905 -18-16-1	46 +2 +4 Pd 106.4 -18-18-0	47 +1 Ag 107.870 -18-18-1	48 +2 Cd 112.40 -18-18-2	49 +3 In 114.82 -18-18-3	50 +2 +4 Sn 118.69 -18-18-4	51 +3 +5 −3 Sb 121.75 -18-18-5	52 +4 +6 −2 Te 127.60 -18-18-6	53 +1 +5 +7 −1 I 126.9044 -18-18-7	54 0 Xe 131.30 -18-18-8	-M-N-O
55 +1 Cs 132.905 -18-8-1	56 +2 Ba 137.34 -18-8-2	57* +3 La 138.91 -18-9-2	72 +4 Hf 178.49 -32-10-2	73 +5 Ta 180.948 -32-11-2	74 +6 W 183.85 -32-12-2	75 +4 +6 +7 Re 186.2 -32-13-2	76 +3 +4 Os 190.2 -32-14-2	77 +3 +4 Ir 192.2 -32-15-2	78 +2 +4 Pt 195.09 -32-16-2	79 +1 +3 Au 196.967 -32-18-1	80 +1 +2 Hg 200.59 -32-18-2	81 +1 +3 Tl 204.37 -32-18-3	82 +2 +4 Pb 207.19 -32-18-4	83 +3 +5 Bi 208.980 -32-18-5	84 +2 +4 Po (210) -32-18-6	85 At (210) -32-18-7	86 0 Rn (222) -32-18-8	-N-O-P
87 +1 Fr (223) -18-8-1	88 +2 Ra (226) -18-8-2	89** +3 Ac (227) -18-9-2																-O-P-Q

*Lanthanides	58 +3 +4 Ce 140.12 -19-9-2	59 +3 Pr 140.907 -20-9-2	60 +3 Nd 144.24 -22-8-2	61 +3 Pm (145) -23-8-2	62 +2 +3 Sm 150.35 -24-8-2	63 +2 +3 Eu 151.96 -25-8-2	64 +3 Gd 157.25 -25-9-2	65 +3 Tb 158.924 -26-9-2	66 +3 Dy 162.50 -28-8-2	67 +3 Ho 164.930 -29-8-2	68 +3 Er 167.26 -30-8-2	69 +3 Tm 168.934 -31-8-2	70 +2 +3 Yb 173.04 -32-8-2	71 +3 Lu 174.97 -32-9-2	-N-O-P
**Actinides	90 +4 Th 232.038 -19-9-2	91 +5 +4 Pa (231) -20-9-2	92 +3 +4 +5 +6 U 238.03 -21-9-2	93 +3 +4 +5 +6 Np (237) -22-9-2	94 +3 +4 +5 +6 Pu (242) -23-9-2	95 +3 +4 +5 +6 Am (243) -24-9-2	96 +3 Cm (247) -25-9-2	97 +3 +4 Bk (249) -26-9-2	98 +3 Cf (251) -28-8-2	99 Es (254) -29-8-2	100 Fm (253) -30-8-2	101 Md (256) -31-8-2	102 (254) -32-8-2	103 Lw	-O-P-Q

Numbers in parentheses are mass numbers of most stable isotope of that element.

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* Adapted from Handbook of Chemistry and Physics, 47th Edition, The Chemical Rubber Company, Cleveland, Ohio. Values for atomic weights given in this table may differ slightly from the values listed in Section 1.

ELECTRON CONFIGURATION OF PERIODS 1, 2, and 3 FROM THE PERIODIC CHART

Atomic Number	*Element*	*K*	*L*	*M*	
1	Hydrogen	1			
2	Helium	2			Inert
3	Lithium	2	1		
4	Beryllium	2	2		
5	Boron	2	3		
6	Carbon	2	4		
7	Nitrogen	2	5		
8	Oxygen	2	6		
9	Fluorine	2	7		
10	Neon	2	8		Inert
11	Sodium	2	8	1	
12	Magnesium	2	8	2	
13	Aluminum	2	8	3	
14	Silicon	2	8	4	
15	Phosphorus	2	8	5	
16	Sulfur	2	8	6	
17	Chlorine	2	8	7	
18	Argon	2	8	8	Inert

APPENDIX B. TABLE OF CLINICAL VALUES*

Section 1. *Analyses Performed on Serum (unless otherwise specified)*

ANALYSIS	*NORMAL*	*SPECIAL INSTRUCTIONS*
Acetone plus acetoacetate	0.3 to 2 mg.%	
Albumin	See under protein	
Alcohol	See ethanol	
Ammonia	40 to 70 µg.%	Use heparinized syringe.
Amylase	Varies with test	
Ascorbic Acid	0.4 to 1.5 mg.%	Very unstable. Decreases even at low temperatures, and disappears within hours at room temperature.
Bilirubin	Direct: 0.2 mg.% Total: 0.6 mg.%	Hemolysis decreases.
Bromsulfalein (BSP)	Less than 5% retention in 45 min.	
BUN	See urea nitrogen	
Calcium	9 to 11 mg.%	BSP dye interferes.
Carbon dioxide (plasma may be preferred)	26 to 28 mEq/L. Infants lower	Heparinize syringe and store in suitable plastic lined container if to be shipped.

*Values for newborn infants may be found in *The Physiology of the Newborn Infant*, Smith, C. A., 3rd Ed. 1959, Charles C. Thomas Co.

ANALYSIS	*NORMAL*	*SPECIAL INSTRUCTIONS*
Carbon monoxide	Symptomatic at 20% saturation or less	Use whole blood. Li Oxalate acceptable. Stopper tightly.
Carotene	1 to 3 units per ml.	Variation among sources in value of normals.
Chloride	98 to 106 mEq/L.	
Cholesterol	Total 150 to 310 mg.% Esters 60 to 80% tot.	Bilirubin will elevate.
Copper	100 to 200 µg. per 100 ml.	Ship in bottle which is acid-washed and rinsed in copper-free water.
Creatinine	0.7 to 1.5 mg.%	
Cryoglobulins	None	Keep at 37°C. until serum is separated.
Ethanol (whole blood)	Subject to interpretation	Lithium Oxalate; stopper tightly; refrigerate.
Glucose (whole blood preferred)	80 to 120 mg.% total reducing substance	Sodium fluoride recommended preservative, or send PFF if shipped.
Hemoglobin electrophoresis (cells)		Wash cells three times with saline, add equal amt. dist. water and 1 ml. toluene.

ANALYSIS	*NORMAL*		*SPECIAL INSTRUCTIONS*
Hemoglobin, plasma	Less than 5 mg.%		Avoid hemolysis 1 ml. 3% sodium citrate recommended anticoagulant per 10 ml. of blood.
Iodine, Protein Bound	See PBI		
Iron	50 to 150 µg. per 100 ml.		Lower in AM (diurnal variation).
Iron-binding capacity			non-icteric fasting specimen.
	Male	*Female*	
1. Unsaturated iron-binding capacity	0.150 to 0.222 mg.%	0.144 to 0.322 mg.%	
2. Total iron-binding capacity	0.254 to 0.432 mg.%	0.224 to 0.415 mg.%	
Lactic dehydrogenase	Units vary with procedure		Must not be hemolyzed.
Magnesium	1.5 to 2.5 mEq/L.		
Methemoglobin (whole blood)	Zero		Avoid excessive oxalate.
PBI	4 to 8 µg.%		Avoid iodine contamination.
Phenylalanine	0 to 2 mg.%		
Phosphatase, acid	Units vary with procedure		Freeze if to be stored; avoid hemolysis. Minimize storage time.
Phosphatase, alkaline	Units vary with procedure		BSP dye interferes.

ANALYSIS	*NORMAL*	*SPECIAL INSTRUCTIONS*
Phosphorus, inorganic	3.0 to 4.5 mg.% children higher	Use fasting specimen and separate serum as soon as possible. Minimize storage time.
Potassium	3.8 to 5.0 mEq/L.	Serum must be separated from cells within 1 hr.
Protein:		
Total	6 to 8 g. per 100 ml.	
Albumin	3.6 to 5.6 g.%	
Globulin	1.3 to 3.2 g.%	
A/G ratio	1.5 to 2.5:1	
Protein by paper electrophoresis:		
Albumin	45 to 55% of total	
Globulin, Alpha$_1$	5 to 8%	
Alpha$_2$	8 to 13%	
Beta	11 to 17%	
Gamma	15 to 25%	
Protein, Fibrinogen (whole blood)	200 to 600 mg.%	Use K-Oxalate only; some methods use whole blood directly into buffer soln.
Salicylate	To 30 mg.% aver. therapeutic maximum dose	Serum, plasma, or PFF; toxicity is a matter of clinical interpretation.
Sodium	138 to 146 mEq/L.	
Transaminase	SGOT and SGPT Units vary with procedure	

ANALYSIS	*NORMAL*	*SPECIAL INSTRUCTIONS*
Urea Nitrogen	8 to 25 mg.%	Do not use ammonium oxalate. Range will be more restricted with certain methods.
Uric Acid	4 to 6 mg.%	Separate cells and serum. Serum should be clear.
Vitamin A	0.5 to 2.0 units/ml.	Texts vary in defining what is normal.

Section 2. *Analyses Performed on Urine*

ANALYSIS	*NORMAL*	*SPECIAL INSTRUCTIONS*
Acetone plus acetoacetate	None	Refrigerate specimen.
Coproporphyria	50 to 250 µg./day; Children less	24-hr. specimen with 5 g. sodium carbonate.
Creatine	0 to 200 mg./24 hr. Usually less than 100 mg./24 hr.	Accomplished in conjunction with creatinine.
Homogentisic acid	None	Refrigerate 24-hr. urine. Salicylates interfere.
5-HIAA	Zero	Collect with HAc in container. pH must be below 6 at all times.

ANALYSIS	*NORMAL*			*SPECIAL INSTRUCTIONS*
Lead	Less than 120 µg./24 hr.			Collect in lead-free container in lead-free environment.
PSP	25% by 15 min. 40% by 30 min. 60% by 2 hr.			BSP interferes.
Porphobilinogen	Zero			Use freshly voided specimen.
Protein	None			
Steroids:	*Age*	*Male*	*Female*	
17-keto	10	1 to 4 mg.	1 to 4 mg.	Meprobamate interferes. Values are for 24-hr. specimens.
	20	6 to 21	4 to 16	
	30	8 to 26	4 to 14	
	50	5 to 18	3 to 9	
	70	2 to 10	1 to 7	
17-hydroxy	Males 2 to 7 mg./24 hr.; females lower.			Chlorpromazine interferes.
Urobilinogen	Less than 1 Ehrlich unit.			2-hr. PM specimen. Usually 1300 to 1500 hr.
Vanilmandelic acid (VMA)	Less than 9 mg./24 hr.			Collect with 12 ml. concentrated HCl so pH remains 2 to 3.

Section 3. *Collection and Shipment of Urine Specimens*

Procedure or Component	*Volume Required*	*Collection and Shipment*
δ-Aminolevulinic acid (ALA)	50 ml. aliquot	Collect in dark brown bottle containing not less than 10 ml. of conc. HCl.* When possible, ship frozen with no additives.
Coproporphyrins	50 ml.	Collect in dark brown bottle containing 2 to 5 g. sodium carbonate. (Toluene may also be added or keep refrigerated.) Ship frozen.
17-Hydroxycorticosteroids (17OHCS)	50 ml. aliquot of 24-hr. specimen	Add 0.4 g. boric acid to 50 ml. aliquot.
5-Hydroxyindoleacetic acid (5HIA)	50 ml. aliquot of 24-hr. specimen	Collect with 10 to 15 mL glacial acetic acid in container.
Nitrogen, Alpha amino	50 ml. aliquot of 24-hr. specimen	Add 1 ml. conc. HCl to 50 ml. aliquot.
Porphobilinogen	50 ml. aliquot of 24-hr. specimen	See note under δ-aminolevulinic acid.
Porphyrins, quantitative	50 ml. aliquot of 24-hr. specimen	See note under coprophyrins.

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*On quantitative studies, always record and forward the 24-hour volume figure to the reference laboratory.

Procedure or Component	*Volume Required*	*Collection and Shipment*
Proteins (Electrophoresis)	50 ml. aliquot of 24-hr. specimen	Collect under 5 or 10 ml. of toluene. Remove the latter before shipment. Add 50 mg. of sodium azide per 50 ml. aliquot.
Vanylmandelic acid (VMA)	50 ml. aliquot of 24-hr. specimen	Collect with 12 ml. concentrated HCl or 15 ml. concentrated glacial acetic acid so pH remains 2 to 3; specimen must not be collected without the acid.

Section 4. *Analyses Performed on Spinal Fluid*

ANALYSIS	*NORMAL*
Chloride	120 to 128 mEq/L.
Glucose	50 to 75 mg.%
Protein:	
Lumbar	15 to 45 mg.%
Cisternal	15 to 25 mg.%
Ventricular	5 to 15 mg.%

Section 5. *Bibliography to Appendix B*

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APPENDIX C. MATHEMATICS

Section 1. *Equations*

An equation is a mathematical expression of equalities. In clinical chemistry computations, it is often necessary to transpose members of an equation in order to solve for an unknown. This section explains some of the mechanisms involved in solving equations. Consider the following example of an equation.

$$\frac{OD_u}{OD_s} \text{ X } C_s \text{ X } \frac{100}{V} = C_u$$

Whatever mathematical operation is performed on the left side of the equation must be performed on the right side to avoid disturbing the equality. For example, each side could be multiplied by 100 and the expression would still hold true. If all the values except C_u are known, it involves no more than simple arithmetic to solve for C_u. But if all of the values except OD_s are known, it is necessary to get OD_s alone on one side of the equal sign. The way this is accomplished in algebra is to perform a mathematical procedure opposite to that expressed on the side of the equation in which the quantity appears, in this case OD_s. Note that in the original equation OD_s is divided out on the left side; hence multiplying both sides of the equation by OD_s will cause it to appear on the right side as follows.

$$OD_u \text{ X } C_s \text{ X } \frac{100}{V} = C_u \text{ X } OD_s$$

Now divide both sides by C_u, since a multiplication is expressed on the right side of the equation. The equation then becomes

$$\frac{OD_u \text{ X } C_s \text{ X } 100}{V \text{ X } C_u} = OD_s$$

and is said to be expressed in terms of OD_s. The reason for moving OD_s to the right side of the equation is to get it alone on one side with minimum effort. This could also have been accomplished by the following steps.

Step 1: Divide each side by C_s.

Step 2: Multiply both sides by $\frac{V}{100}$.

Step 3: Divide both sides by OD_u.

Step 4: Invert both sides, since OD_s now appears as $\frac{1}{OD_s}$ on the left side as a result of dividing out OD_u.

Note that regardless of how you solve for the unknown, a factor of the unknown is always eliminated by a mathematical procedure opposite to that expressed in the equation. Consider the equation $x + 20 = y$. In order to solve for x, subtract 20 from both sides, because subtraction is the opposite of the addition expressed on the left side of the equation. The result would be $x = y - 20$. It must be remembered that although multiplication and division can be accomplished in any order, one cannot multiply or divide across plus or minus signs. Consequently, expressions such as $y - 20$ should be placed in parentheses and written $(y - 20)$ if there are other factors of that expression. For example, the equation

$x = \frac{y - 20}{10}$ is best written $x = \frac{(y - 20)}{10}$ to emphasize this expression is a quantity and *not* equivalent to $\frac{(y - 2)}{1}$. It makes no difference in which factor of an equation multiplication or division is expressed. For example

$$\frac{OD_u}{OD_s} \text{ X } C_s \text{ X } \frac{100}{V}$$

is the same as

$$OD_u \text{ X } \frac{C_s}{V} \text{ X } \frac{100}{OD_s} \quad \text{or} \quad \frac{OD_u \text{ X } C_s \text{ X } 100}{OD_s \text{ X } V}$$

If V is equal to 100, the factor of 100 in the numerator would cancel out, which is not the case when the numerator involves addition $(OD_u + C_s + 100)$ rather than multiplication.

The fundamental operations of mathematics may be summarized in three laws. 1. *The commutative law* states that regardless of the order of addition or multiplication, the result is the same. Example: $x + y = y + x$. 2. *The associative law* states that the product of three or more factors and the sum of three or more terms is the same regardless of the manner in which they are grouped. Example: $x + (y + z) = (x + y) + z = x + y + z$ and $x(yz) = (xy)z = xyz$. 3. *Distributive law* states that two or more terms multiplied by a single factor is equal to the sum of the products of each term of the expression multiplied by the single factor. Example: $a(x+y-z) = ax + ay - az$.

Section 2. *Exponents*

An exponent is a term written above and to the right of another quantity, denoting how many times the latter is repeated as a factor: $2^3 = (2)(2)(2) = 8$. It is read as the *power* of the number; thus, 2^3 is read "two to the third power" or simply "the cube of two." A number raised to a power is referred to as an *exponential number*. The exponent 1 is not indicated in that it does not change the value of the quantity: $3^1 = 3$. The *exponent* is called the *power* and the number is called the *base*. Thus, for a^x the exponent is x and the base is a.

Addition and subtraction. When adding or subtracting quantities with exponents, the exponent does not change:

$$2a^2 + a^2 = 3a^2 \qquad 2a^2 - a^2 = a^2$$

The coefficient 1 is understood in the case of a^2, since $1 \times a^2 = a^2$.

Multiplication. The first law of exponents states that the exponent of any number in a product equals the sum of its exponents in the factors of the product:

$$x^7 = (x^3)(x^4) \qquad y^{x+2} = (y^x)(y^2)$$

(1) To multiply two like quantities, each raised to some power, the exponents are added and the common quantity is retained:

$$(x^3)(x^2) = (x)(x)(x)(x)(x) = x^5$$

(2) When unlike quantities, each raised to a power, are multiplied, the results of each quantity must first be determined or the quantities must be expressed by a common base:

$$(4^2)(3^3) = (16)(27) \qquad (4^2)(2^2) = (2^4)(2^2) = 2^6$$

Division. To divide two like quantities, each raised to some power, the exponents are subtracted and the common quantity is retained:

$$\frac{x^5}{x^2} = \frac{(x)(x)(x)(x)(x)}{(x)(x)} = x^{(5-2)} = x^3$$

Negative exponents. A negative exponent denotes division: $3^{-2} = \frac{1}{3^2}$. Hence, a quantity raised to a negative power is the reciprocal of the quantity to a positive power. *The second law of exponents* states that the exponent of any number in a quotient equals its exponent in the dividend minus its exponent in the divisor:

$$\frac{3^2}{3^4} = \frac{1}{3^2} = 3^{-2} \qquad \frac{y^x}{y^z} = y^{x-z}$$

Powers. *The third law of exponents* states that any exponential quantity raised to a power is equal to the quantity raised to the product of the exponent and the power of the exponential quantity:

$$(3^2)^3 = 3^6 \qquad (y^x)^n = y^{(x)(n)}$$

(1) Any quantity, except zero, raised to the zero power is 1; $6^0 = 1$. This relation of the zero power to any quantity is clearly seen by taking the number to the power of 1 and -1:

$$6^1 = 6 \text{ and } 6^{-1} = \frac{1}{6}$$

$$(6^1)(6^{-1}) = 6^{(1-1)} = 6^0 = \frac{6^1}{6^1} = 1$$

$$10^1 = 10,\ 10^{-1} = \frac{1}{10}, \text{ and } 10^0 = 1$$

(2) When a product is raised to a power, each member is raised to that power:

$$(2xy)^4 = 16x^4y^4$$

(3) To find the result of an expression such as $2x^3$, first raise the term to the power indicated, then multiply the quantity by the coefficient:

If $x = 3$,

$$2 \times 3^3 = 2 \times 27 = 54$$

Roots. A root indicates that a quantity is raised to a fractional power:

$$\sqrt[2]{16} = 16^{\frac{1}{2}}$$

The fourth law of exponents states that the radicand is expressed as an exponential quantity to a power equal to the exponent divided by the index of the root:

$$\sqrt[2]{4^{3}} = 4^{\frac{3}{2}} \qquad \sqrt[n]{y^{x}} = y^{\frac{x}{n}}$$

The symbol indicating a root is a *radical sign* ($\sqrt{\ }$). When a radical sign appears in an expression, the expression is a *radical* ($2\sqrt{5}$), the number above the radical sign is the *index* ($\sqrt[3]{\ }$), and the number under the radical sign is the *radicand* ($\sqrt{7}$). The absence of an index above the radical sign indicates an index of 2, or simply the square root.

(1) To raise a radical to a power, find the root and raise it to the power indicated by applying the third law of exponents:

$$(\sqrt{4^{3}})^{4} = (4^{\frac{3}{2}})^{4} = 4^{6} \qquad \left(\sqrt[n]{y^{x}}\right)^{a} = \left(y^{\frac{x}{n}}\right)^{a} = y^{\frac{ax}{n}}$$

(2) The root of a root is taken by reducing the dual radical to the power of the radicand:

$$\sqrt{\sqrt[3]{2}} = \left(\sqrt[3]{2}\right)^{\frac{1}{2}} = 2^{\frac{1}{6}}$$

(3) Addition or subtraction of quantities within radicals is possible only when the quantities and indices are similar. Thus,

$$\sqrt{2} + \sqrt{2} = 2\sqrt{2}$$

but

$$\sqrt[3]{2} + \sqrt{2}$$

cannot be further combined without changing their forms. For example:

$$\sqrt{8} + \sqrt{2} = \sqrt{(4)(2)} + \sqrt{2} = 2\sqrt{2} + \sqrt{2} = 3\sqrt{2}$$

(4) Multiplication can be performed directly when radicals have the same index:

$$(2\sqrt{x})(3\sqrt{y}) = 6\sqrt{xy}$$

When indices are dissimilar, the radical is changed to exponential form and the product found is returned to radical form:

$$(\sqrt{3})(\sqrt[3]{3}) = (3^{\frac{1}{2}})(3^{\frac{1}{3}}) = 3^{\frac{1}{2}+\frac{1}{3}} = 3^{\frac{5}{6}} = \sqrt[6]{3^{5}}$$

(5) Division of quantities within a radical sign or of a radical by a radical, can be performed by changing to exponential form:

$$\sqrt{\frac{9}{16}} = \frac{\sqrt{9}}{\sqrt{16}} = \left(\frac{9}{16}\right)^{\frac{1}{2}} = \frac{9^{\frac{1}{2}}}{16^{\frac{1}{2}}} = \frac{3}{4}$$

Section 3. *Logarithms*

A logarithm is an exponent. The *logarithm* of a number, to any base, is the power to which the base must be raised to equal the given number. That is, if $N = a^x$, the logarithm of N to the base a is x, or $\log_a N = x$. From this definition, a logarithm is an exponent and all the index laws of exponents apply to logarithms. The two statements $N = a^x$ and $\log_a N = x$ express the same relationship, being in exponential and logarithmic form, respectively. Thus:

If $2^2 = 4$, the logarithm of 4 to the base 2 is 2 and $\log_2 4 = 2$

If $5^3 = 125$, then $\log_5 125 = 3$

A negative number has no true logarithm.

Systems of Logarithms. There are two systems of logarithms in use: the *common* (Briggsian) *system* to the base 10 and the *natural* (hyperbolic) *system* to the base e (2.71828). Common logarithms are written as $\log_{10} N$ or simply $\log N$ and natural logarithms as $\log_e N$ or $\ln N$. The two systems are interrelated as follows:

$$\log N = 0.4343 \ln N \qquad (2\text{-}1)$$

$$\ln N = 2.3026 \log N \qquad (2\text{-}2)$$

Common Logarithms. Common logarithms consist of an integral part called the *characteristic* and a decimal part called the *mantissa*.

o *Characteristic.* The characteristic of any number is determined by the position of the decimal point within that number. When the number is greater than 1, the numerical value of the characteristic is one less than the number of significant figures to the left of the decimal point; when the number is less than 1, the characteristic is negative and is one greater than the number of zeros immediately to the right of the decimal point.

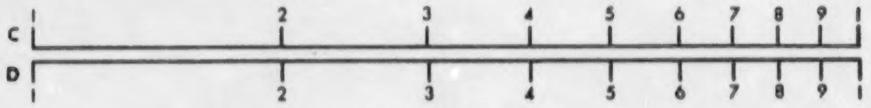
o *Mantissa.* An important consequence of the use of the base 10 is that the mantissa of the logarithm is independent of the decimal point. The numbers 149,000, 149 and 0.149 all have the same mantissa.

Section 4. *Review of the Slide Rule.*

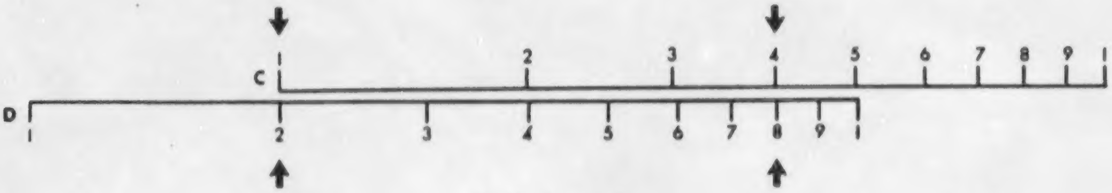
This section on the use of the slide rule is a review of some important rules applicable to the solution of problems in this course. It is not intended as a complete course of instruction.

Multiplication and division with the slide rule are accomplished by use of the *C* and *D* scales, each of which is identically scaled in logarithmic lengths. Thus, multiplication and division are actually the physical addition and subtraction of the logarithms of the numbers on the slide rule. Squares and square roots are found by use of the *A* and *D* scales; cubes and cube roots, by use of the *K* and *D* scales.

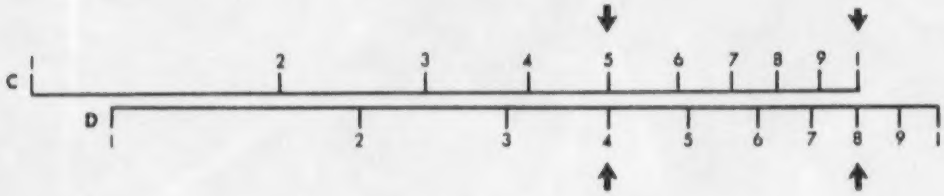
Multiplication. To multiply two numbers, place either the right or left index of the *C* scale opposite the first factor on the *D* scale; and read the result on *D* directly under the second factor on *C* using the hairline on the indicator. (Use the index which keeps the second factor on *C* within the range of *D*.)



SLIDE RULE

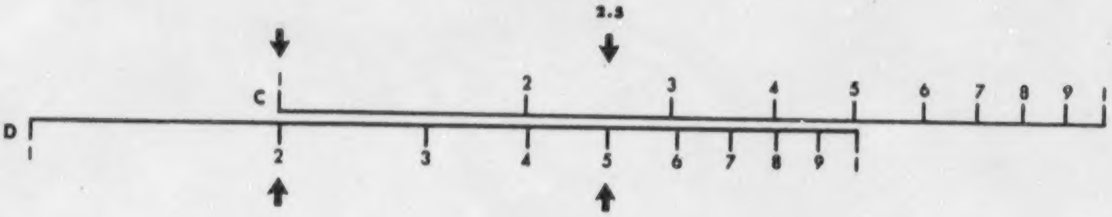


2 X 4 = 8

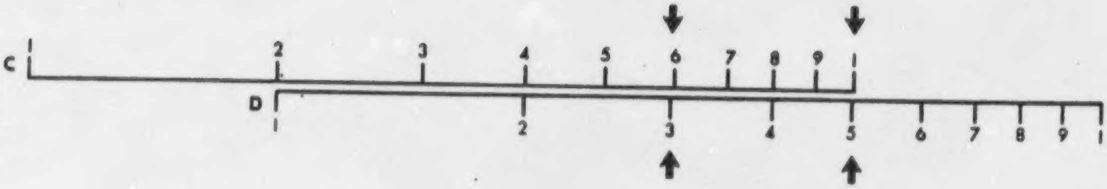


5 X 8 = 40

Division. Division with the slide rule is the reverse of multiplication. To divide one number by another, place the hairline of the indicator over the dividend on *D*, move the divisor on *C* to the hairline, and directly under the index of *C* read the quotient on *D*.

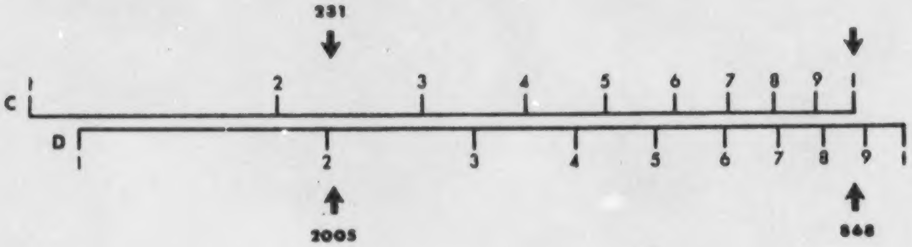


$$\frac{5}{2.5} = 2$$



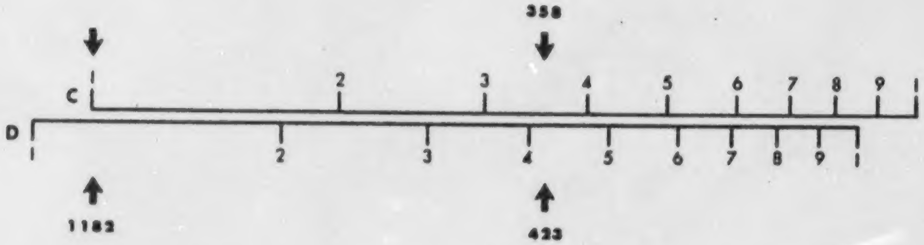
$$\frac{3}{6} = 0.5$$

Decimal point. Determination of the position of the decimal point in slide-rule calculations is facilitated by expressing each factor as a number between 1 and 10 multiplied by a power of 10. For example, $420 = 4.2 \times 10^2$. Similarly, if 8,682.3 is to be multiplied by 0.0231, the two factors may be expressed as 8.68×10^3 and 2.31×10^{-2}. The significant figures are then multiplied on the slide rule. The decimal point for the product of 2.31×8.68 is easily determined by inspection; the exponents of 10 are added: $(10)^3 \times (10)^{-2} = 10^{3-2} = 10$.


231
C
D
1
2
3
4
5
6
7
8
9
2005
868

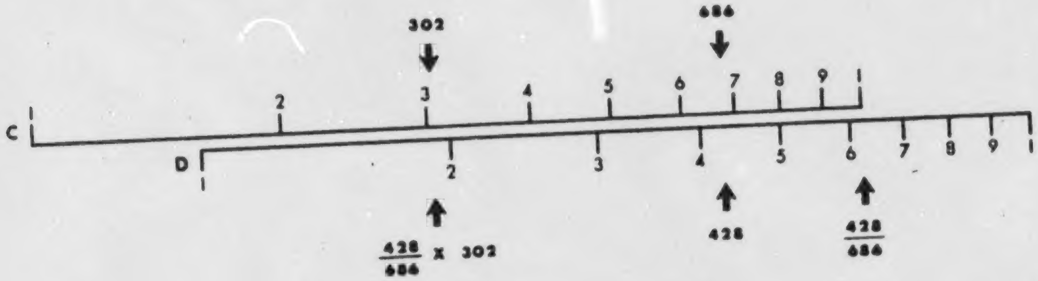
$$(8.68 \times 10^3) \times (2.31 \times 10^{-2}) = 20.05 \times 10 = 2.005 \times 10^2$$

In division, the decimal point of the quotient read from the slide rule is also determined by inspection, and the exponent of 10 in the divisor is subtracted from the exponent of 10 in the dividend (law of exponents). For example, in division of 42,301 by 0.0358, the figures are expressed in powers of 10 and divided as shown.


358
C
D
1
2
3
4
5
6
7
8
9
1182
423

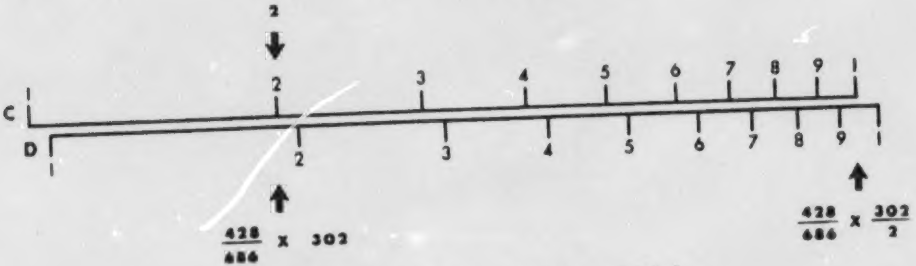
$$\frac{4.23 \times 10^4}{3.58 \times 10^{-2}} = 1.182 \times 10^6$$

Multiple operations. Any number of factors can be handled with the slide rule. For example, to calculate $\frac{428 \times 302}{686 \times 2}$, first express as $\frac{4.28 \times 10^2 \times 3.02 \times 10^2}{6.86 \times 10^2 \times 2}$, then place 6.86 on *C* over 4.28 on *D*. The index is now over the quotient. Thus, under 3.02 on *C* lies the answer to $\frac{4.28}{6.86} \times 3.02$. By placing the hairline on 3.02 and drawing 2 on *C* to the hairline, the final answer is found on *D* under the index of *C*, as shown below.


302
686
C
D
2
3
4
5
6
7
8
9
1
$\frac{428}{686}$ x 302
428
$\frac{428}{686}$

First Setting of the Slide

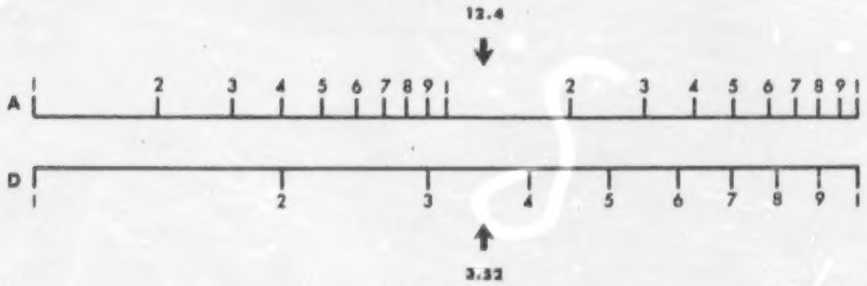
$$\frac{4.28 \times 10^2 \times 3.02 \times 10^2}{6.86 \times 10^2}$$


2
C
D
2
3
4
5
6
7
8
9
1
$\frac{428}{686}$ x 302
$\frac{428}{686}$ x $\frac{302}{2}$

Second Setting of the Slide

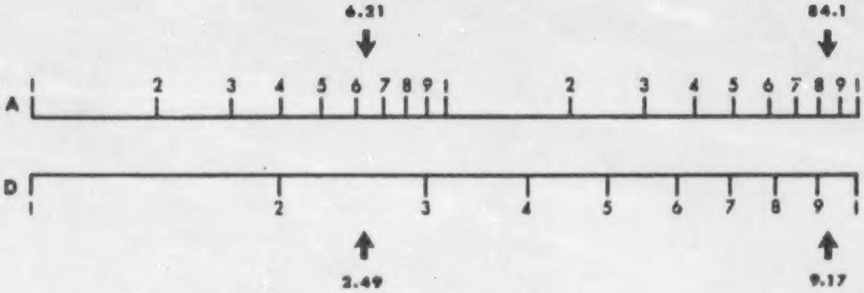
$$\frac{4.28 \times 10^2 \times 3.02 \times 10^2}{6.86 \times 10^2 \times 2} = 0.944 \times 10^2 = 9.44 \times 10^1 = 94.4$$

Squares and square roots. Squares and square roots are calculated by use of the *A* and *D* scales. Note that the *A* scale really consists of two *D* scales, each one-half the length of the regular *D* scale. *Thus, the square of every* number on *D* lies directly above it on *A*. Notice 4 on *A* above 2 on *D*; and 9 on *A* above 3 on *D*. Here the use of powers of 10 is advantageous. For example, in finding the square of a number, it may be expressed in terms of a number between 1 and 10, multiplied by a power of 10. On the slide rule, first place the hairline over the number on *D* and read the square directly above on *A*. Then multiply the exponent of 10 by 2 (law of exponents). When using this exponential method with squares and square roots, the *D* scale represents numbers between 1 and 10 and the *A* scale represents numbers between 1 and 100. For example, $(3{,}520)^2$ is calculated below.



$$(3{,}520)^2 = (3.52 \times 10^3)^2 = 12.4 \times 10^6 = 1.24 \times 10^7$$

In extracting the square root of a number, it is expressed in the form of a number between 1 and 100, multiplied by 10 to an even power. On the slide rule, first place the hairline over the number on *A* and read its root on *D*. Then divide the exponent of 10 by 2 (law of exponents). For example, to extract the square root of 124,000, express it as 12.4×10^4. Under 12.4 on *A*, read 3.52 on *D*. Divide the exponent of 10 by 2. The answer is 3.52×10^2. To extract the square root of decimal fractions, the method is the same.


6.21
84.1
A
1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9 1
D
1 2 3 4 5 6 7 8 9 1
2.49
9.17

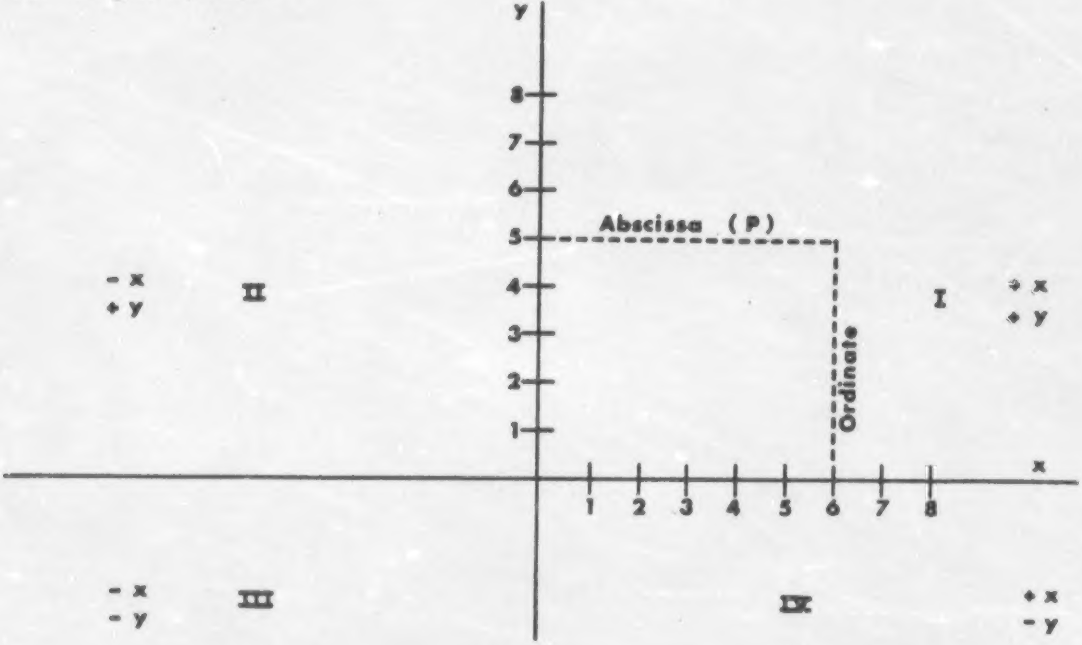
$$\sqrt{0.0000841} = \sqrt{84.1 \times 10^{-6}} = 9.17 \times 10^{-3}$$

$$\sqrt{0.000621} = \sqrt{6.21 \times 10^{-4}} = 2.49 \times 10^{-2}$$

Section 5. *Graphs*

The graph is a pictorial representation of a mathematical equation and may range from a simple straight line to a complicated curve. Graphs are based on the principle of representing a number by a linear distance to a selected scale.

The relationship between two variables is represented by use of two *coordinate axes* perpendicular to each other, the intersection of which is called the *origin*. Normally, the horizontal line is called the x-axis, and the vertical line, the y-axis. The coordinate axes form four *quadrants* which are numbered counterclockwise, I, II, III, and IV, as shown in the figure below.


y
8
7
6
5
4
3
2
1
Abscissa (P)
Ordinate
x
1 2 3 4 5 6 7 8
- x
+ y
II
I
+ x
+ y
- x
- y
III
IV
+ x
- y

The distances of a given point, from the y- and x-axes are called the *abscissa* and *ordinate*, respectively, and are referred to as the *coordinates* of the point. The point P is written as (6,5) where 6 and 5 are the perpendicular intercepts on the x- and y-axes respectively.

Abscissas measured to the *right* of the y-axis are positive while those measured to the *left* are negative; ordinates measured *up* from the x-axis are positive while those measured *down* are negative. You will probably not be dealing with negative values in the clinical laboratory, but you should understand how any value can be expressed graphically.

Section 6. *Summary of General Formulae*

This section provides a list of general formulae useful in clinical chemistry calculations.

1 PURPOSE: To determine the number of grams of solution required to prepare a W/V percent solution of a specific volume.

EQUATION: Grams of Solute Required Equals:

$$\frac{(\text{percent W/V}) \times (\text{volume in ml. of final solution})}{100}$$

2 PURPOSE: To determine the number of milliliters of solute required to prepare a V/V percent solution of a specific volume.

EQUATION: Milliliters of Solute Required Equals:

$$\frac{(\text{percent V/V}) \times (\text{volume in ml. of final solution})}{100}$$

---

3 **PURPOSE:** To determine the volume of a concentrated reagent which contains a desired weight of the pure compound in solution.

EQUATION: Milliliters of Solution Containing Required Weight Equals:

$$\frac{\text{g. of concentrated solution required}}{\text{specific gravity of concentrated solution}}$$

---

4 **PURPOSE:** To determine the number of milliliters of a concentrated W/W reagent required to prepare a W/V percent solution of a specific volume.

EQUATION: Milliliters Concentrated Reagent to be Used Equals:

$$\frac{\begin{array}{c}\text{ml. equivalent}\\ \text{to 1 g. (list)}\end{array} \text{ X } \begin{array}{c}\text{percent}\\ \text{W/V desired}\end{array} \text{ X } \begin{array}{c}\text{desired final}\\ \text{volume in ml.}\end{array}}{100}$$

---

5 **PURPOSE:** To determine the weight of a compound required to prepare a solution of a specific molarity and volume.

EQUATION: Grams of Compound Required Equals:

$$\frac{\begin{array}{c}\text{molecular}\\ \text{weight}\end{array} \text{ X } \begin{array}{c}\text{desired}\\ \text{molarity}\end{array} \text{ X } \begin{array}{c}\text{final volume}\\ \text{in ml.}\end{array}}{1000}$$

---

6 **PURPOSE:** To determine the number of mililiters of a concentrated W/W reagent required to prepare a solution of a specific molarity and volume.

EQUATION: Milliliters of Concentrated Reagent to be Used Equals:

$$\frac{\begin{array}{c}\text{molecular}\\ \text{weight}\end{array} \text{ X } \begin{array}{c}\text{desired}\\ \text{molarity}\end{array} \text{ X } \begin{array}{c}\text{final volume}\\ \text{in ml.}\end{array} \text{ X } \begin{array}{c}\text{factor}\\ \text{from list}\end{array}}{1000}$$

---

7 PURPOSE: To determine the weight of a compound required to prepare a solution of a specific normality and volume.

EQUATION: Grams of Compound Required Equals:

$$\frac{\text{molecular weight} \times \text{desired normality} \times \text{final volume in ml.}}{(\text{total positive valence}) \times 1000}$$

---

8 PURPOSE: To determine the number of milliliters of a concentrated W/W reagent required to prepare a solution of a specific normality and volume.

EQUATION: Milliliters Concentrated Reagent to be Used Equals:

$$\frac{\text{molecular weight} \times \text{desired normality} \times \text{final volume in ml.} \times \text{factor from list}}{(\text{total positive valence}) \times 1000}$$

---

9 PURPOSE: To determine the number of milliliters of a concentrated stock solution to be diluted to obtain a more dilute solution.

EQUATION: Milliliters Stock Concentrated Solution to be Diluted Equals:

$$\frac{\text{ml. dilute solution desired} \times \text{concentration of dilute solution}}{\text{concentration of stock solution}}$$

---

10 PURPOSE: To convert an electrolyte concentration from mg. per 100 ml. to mEq./L.

EQUATION. mEq./L. (Electrolyte) Equals:

$$\frac{(\text{mg. per 100 ml.}) \times (10)}{\text{milliequivalent weight}}$$

11 PURPOSE: To determine the exact normality of an acid or base following a titration.

EQUATION: Volume of Acid Used X Normality of Acid Equals:

(volume of base required) X (normality of base)

---

12 PURPOSE: To determine the actual concentration of standard used in visual colorimetric and spectrophotometric analyses.

EQUATION: C_s Equals:

$$\text{amount of standard in 1 ml. of solution} \times \text{no. ml. of this standard solution used in the test}$$

---

13 PURPOSE: To determine the actual volume of specimen used in a chemical analysis.

EQUATION: V Equals:

$$\frac{\text{ml. of specimen used to prepare PFF or ml. of specimen initially diluted} \times \text{ml. of PFF or ml. of diluted specimen actually used in test}}{\text{total volume in which original specimen is diluted, e.g., total volume of specimen and precipitating agents when preparing PFF}}$$

---

14 PURPOSE: To determine the concentration of an analyzed specimen when the spectrophotometer is used and the final volume of the standard and unknown are identical.

EQUATION: C_u Equals:

$$\frac{OD_u}{OD_s} \times C_s \times \frac{100}{V}$$

---

15 PURPOSE: To determine the concentration of an analyzed specimen when the spectrophotometer is used, and the final volumes of the standard and unknown are not the same.

EQUATION: C_u Equals:

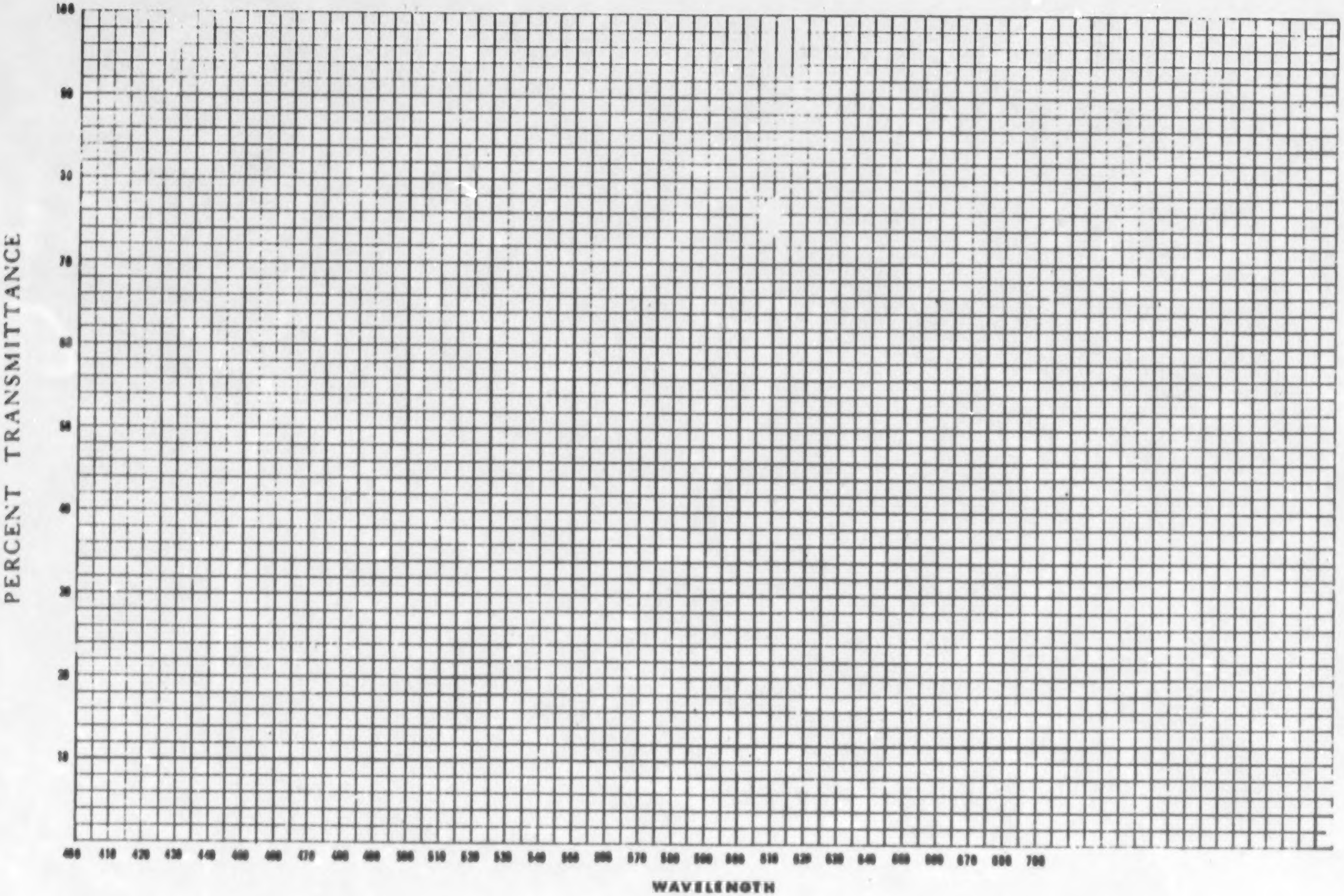
$$\frac{OD_u}{OD_s} \times C_s \times \frac{100}{V} \times \frac{\text{final volume of unknown}}{\text{final volume of standard}}$$

This section provides a summary of general formulae which is useful in clinical chemistry calculations. The following list of reagent factors is required to solve formulae 4, 6, and 8. This factor is the volume of concentrated reagent containing 1 g. of pure reagent. It is equivalent to the reciprocal of the specific gravity of the reagent or $\frac{1}{\text{sp.gr.}}$.

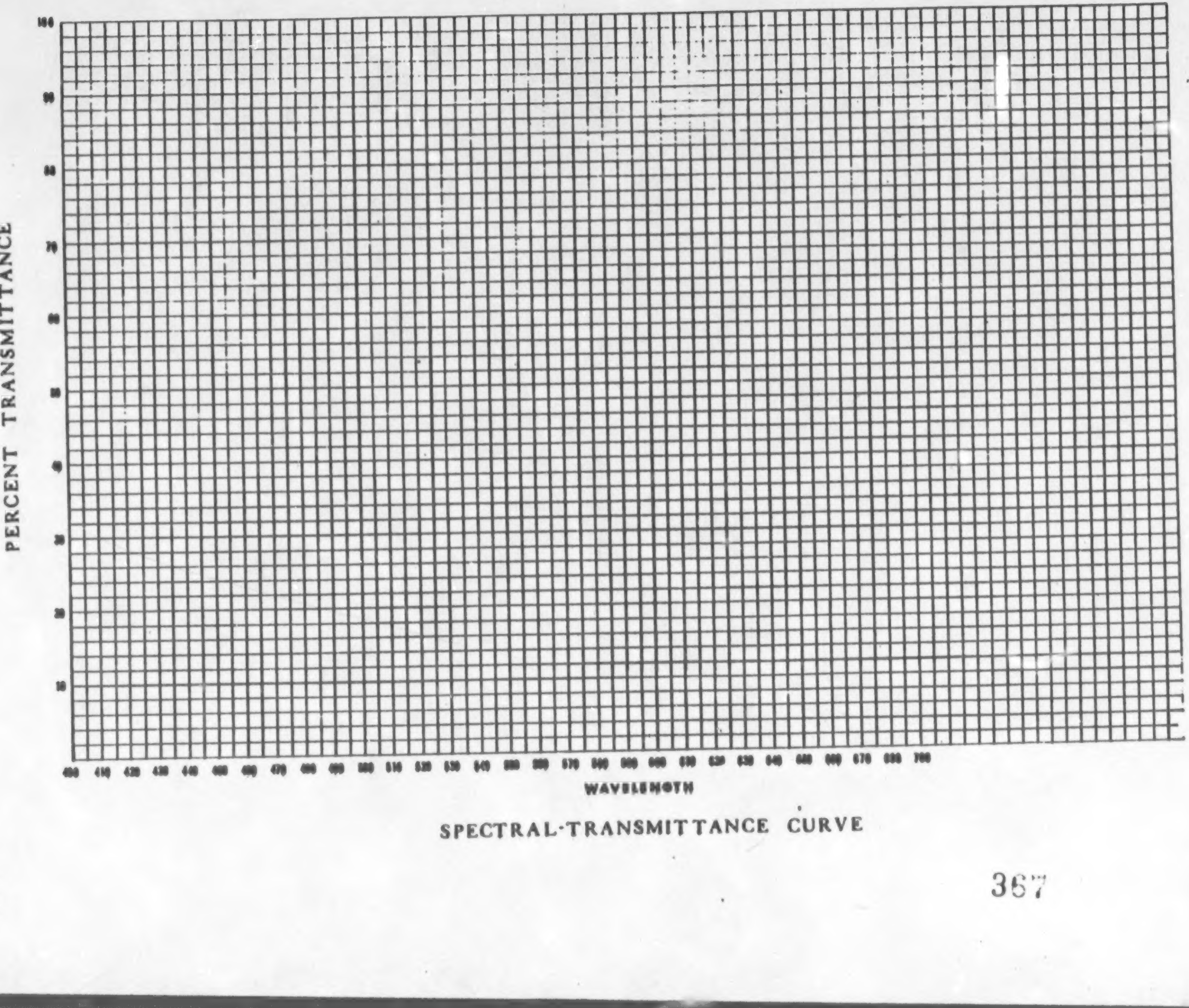
Concentrated Reagent	*Number of ml. that will be approximately equivalent to 1 g. of pure solute*
Acetic acid, glacial......	0.96
Ammonium hydroxide........	3.97
Hydrochloric acid.........	2.30
Nitric acid...............	1.01
Phosphoric acid...........	0.70
Sulfuric acid.............	0.57

Section 7. *Graph Paper*

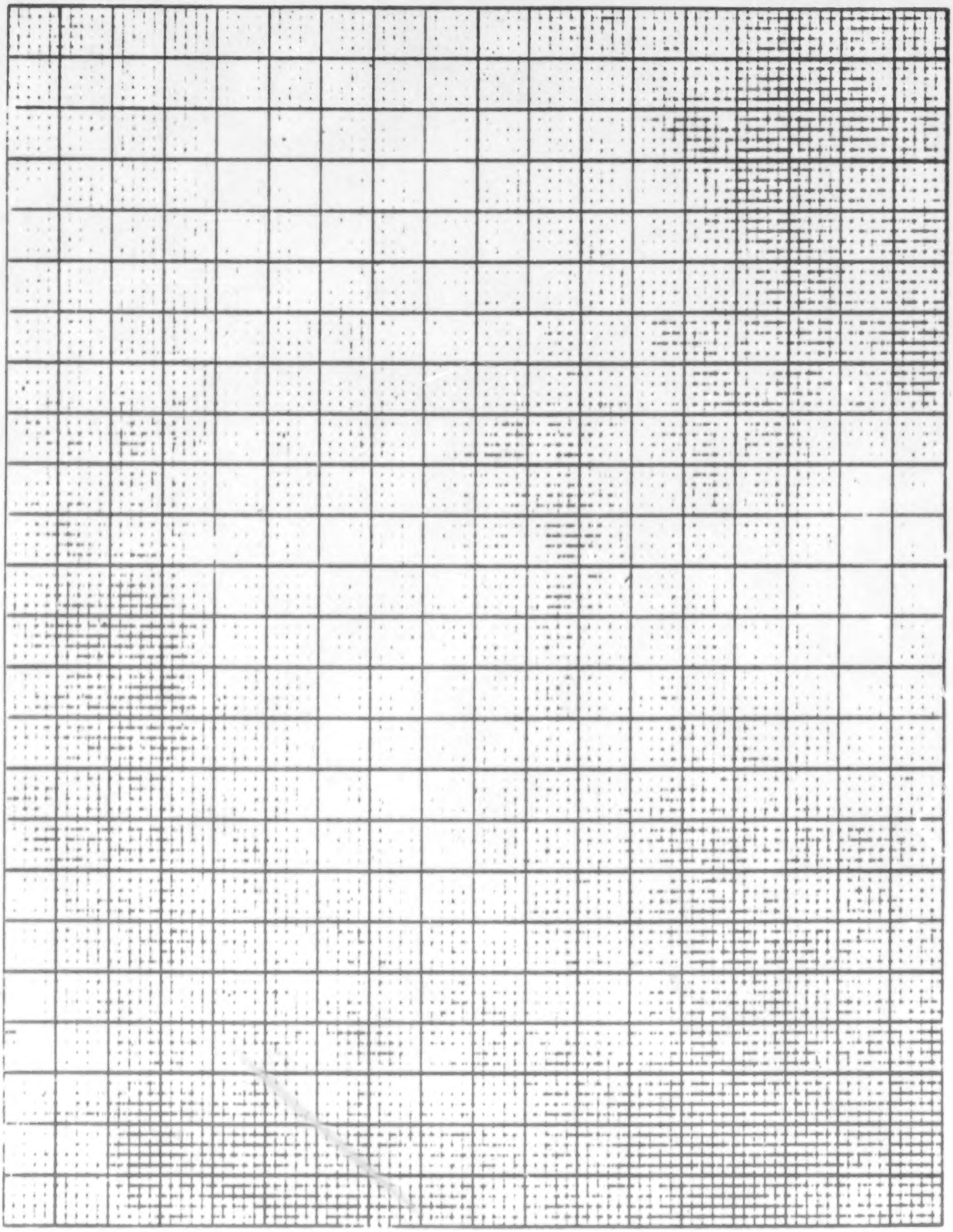
In this section you will find two sheets of graph paper with labeled axes and several sheets of plain graph paper. One of the labeled sheets is required for your solution to Chapter Review Exercise, problem 18, in Chapter 4 of Volume I. One sheet of the plain graph paper is required for your solution to problem situation 4, paragraph 17-12, Chapter 6 of Volume I. The remaining graph paper may be used for your own study and practice. (Remember, do not submit these graphic responses to ECI.)

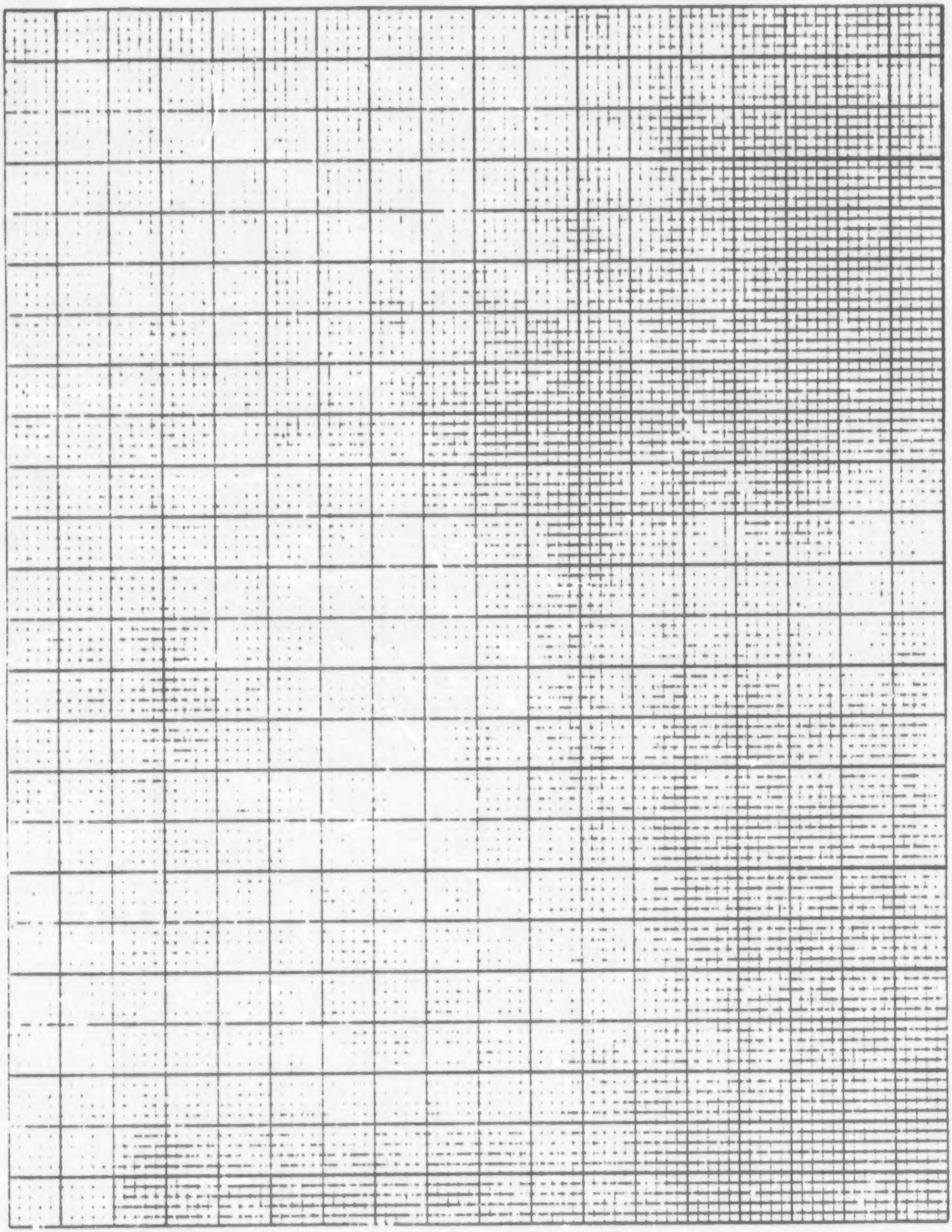


SPECTRAL-TRANSMITTANCE CURVE



SPECTRAL-TRANSMITTANCE CURVE





Section 8. *Standard Deviation Worksheet*

The forms on the following pages are designed for your use in quality control calculations. The procedure is explained in detail in Chapter 6 of Volume I. You will need one sheet to arrange the quality control figures listed in problem situation 4, paragraph 17-12. An extra sheet is included for use in your own laboratory as a guide for arranging quality control information.

Test Results	Differences From Average (d)	Squared Diff. From Average (d^2)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
Mean Ave ($\bar{x}$) =		Sum (Σd^2) =

Calculation of

Standard Deviation

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

$$\sigma = \sqrt{\frac{\quad}{\quad}}$$

$$\sigma = \sqrt{\quad}$$

$\sigma =$

Test Results	Differences From Average (d)	Squared Diff. From Average (d^2)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
Mean Ave ($\bar{x}$) =		Sum (Σd^2) =

Calculation of

Standard Deviation

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

$$\sigma = \sqrt{\frac{\_\_\_\_\_\_}{\_\_\_\_\_\_}}$$

$$\sigma = \sqrt{\_\_\_\_\_\_}$$

$\sigma =$